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	Patents ADP number *(if you know it)*	07157811001
	If the applicant is a corporate body, give the country/state of its incorporation	**ITALY**
4.	Title of the invention	**ANTIGENIC PEPTIDES**
5.	Name of your agent *(if you have one)*	**Carpmaels & Ransford**
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ANTIGENIC PEPTIDES

This invention relates to antigenic peptide sequences from the bacteria *Neisseria meningitidis*.

BACKGROUND

Neisseria meningitidis is a non-motile, gram negative diplococci that are pathogenic in humans.

Based on the organism's capsular polysaccharide, 12 serogroups of *N.meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries.

The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Meningococcus B remains a problem, however. The polysaccharide approach cannot be used because the menB capsular polysaccharide is a polymer of α(2-8)-linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. One approach to a menB vaccine uses mixtures of outer membrane proteins (OMPs) To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed [*eg.* Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28]. Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability [*eg.* Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53].

THE INVENTION

The invention provides fragments of the proteins disclosed in International patent application PCT/IB99/00103 [Annex 1], wherein the fragments comprise at least one antigenic determinant.

Thus, if the length of any particular protein sequence disclosed in PCT/IB99/00103 is *x* amino acids (see Table II), the present invention provides fragments of at most *x-1* amino acids of that protein. The fragment may be shorter than this (*eg. x-2, x-3, x-4, ...*), and is preferably 100 amino

acids or less (*eg.* 90 amino acids, 80 amino acids *etc.*). The fragment may be as short as 3 amino acids, but is preferably longer (*eg.* up to 6, 7, 8, 9, 10, 12, 15, 20, 25, 30, 35, 40, 50, 75, or 100 amino acids).

Preferred fragments comprise the meningococcal peptide sequences disclosed in Table I, or sub-sequences thereof. The fragments may be longer than those given in Table I *eg.* where a fragment in Table I runs from amino acid residue p to residue q of a protein, the invention also relates to fragments from residue (p-*1*), (p-*2*), or (p-*3*) to residue (q+*1*), (q+*2*), or (q+*3*).

The invention also provides polypeptides that are homologous (ie. have sequence identity) to these fragments. Depending on the particular fragment, the degree of sequence identity is preferably greater than 50% (eg. 60%, 70%, 80%, 90%, 95%, 99% or more). These homologous polypeptides include mutants and allelic variants of the fragments. Identity between the two sequences is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty*=12 and *gap extension penalty*=1.

The invention also provides proteins comprising one or more of the above-defined fragments.

The invention is subject to the proviso that it does not include within its scope proteins comprising any of the 45 protein sequences disclosed in PCT/IB99/00103 (*ie.* the even SEQ IDs: 2, 4, 6, 8, 10, ..., 86, 88, 90 of Annex 1).

The proteins of the invention can, of course, be prepared by various means (*eg.* recombinant expression, purification from cell culture, chemical synthesis *etc.*) and in various forms (*eg.* native, C-terminal and/or N-terminal fusions *etc.*). They are preferably prepared in substantially pure form (*ie.* substantially free from other Neisserial or host cell proteins). Short proteins are preferably produced using chemical peptide synthesis.

According to a further aspect, the invention provides antibodies which recognise the fragments of the invention, with the proviso that the invention does not include within its scope antibodies which recognise one of 45 complete protein sequences in Annex I. The antibodies may be polyclonal or, preferably, monoclonal, and may be produced by any suitable means.

The invention also provides proteins comprising peptide sequences recognised by these antibodies. These peptide sequences will, of course, include fragments of the meningococcal proteins in Annex I, but will also include peptides that mimic the antigenic structure of the meningococcal peptides when bound to immunoglobulin.

According to a further aspect, the invention provides nucleic acid encoding the fragments and proteins of the invention, with the proviso that the invention does not include within its scope nucleic acid encoding one of the 45 protein sequences in Annex 1.

In addition, the invention provides nucleic acid comprising sequences homologous (*ie.* having sequence identity) to these sequences. Furthermore, the invention provides nucleic acid which can hybridise to these sequences, preferably under "high stringency" conditions (*eg.* 65°C in a 0.1xSSC, 0.5% SDS solution).

It should also be appreciated that the invention provides nucleic acid comprising sequences complementary to those described above (*eg.* for antisense or probing purposes).

Nucleic acid according to the invention can, of course, be prepared in many ways (*eg.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself *etc.*) and can take various forms (*eg.* single stranded, double stranded, vectors, probes *etc.*). In addition, the term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) *etc.*

According to a further aspect, the invention provides vectors comprising nucleotide sequences of the invention (*eg.* expression vectors) and host cells transformed with such vectors.

According to a further aspect, the invention provides compositions comprising protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as vaccines, for instance, or as diagnostic reagents, or as immunogenic compositions.

The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (*eg.* as vaccines or as immunogenic compositions) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of: (i) a medicament for treating or preventing infection due to Neisserial bacteria; (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised

against Neisserial bacteria; and/or (iii) a reagent which can raise antibodies against Neisserial bacteria. Said Neisserial bacteria may be any species or strain (such as *N.gonorrhoeae*) but are preferably *N.meningitidis*, especially strain A or strain B.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody according to the invention.

According to further aspects, the invention provides various processes.

A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression.

A process for producing protein or nucleic acid of the invention is provided, wherein the protein or nucleic acid is synthesised in part or in whole using chemical means.

A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting said duplexes.

A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

A summary of standard techniques and procedures which may be employed in order to perform ~~the invention (*eg.* to utilise the disclosed sequences for vaccination or diagnostic purposes)~~ follows. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

General

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature *eg.* Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and ii* (D.N Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid*

Hybridization (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press, Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C. C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

All publications, patents, and patent applications cited herein are incorporated in full by reference.

Definitions

A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "comprising" means "including" as well as "consisting" *eg.* a composition "comprising" X may consist exclusively of X or may include something additional to X, such as X+Y.

The term "antigenic determinant" includes B-cell epitopes and T-cell epitopes.

The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a meningococcal sequence is heterologous to a mouse host cell. A further examples would be two epitopes from the same or different proteins which have been assembled in a single protein in an arrangement not found in nature.

An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

Expression systems

The meningococcal nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, baculoviruses, plants, bacteria, and yeast.

i. Mammalian Systems

Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation [Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual, 2nd ed.]*.

Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallotheionein gene, also provide

useful promoter sequences. Expression may be either constitutive or regulated (inducible), depending on the promoter can be induced with glucocorticoid in hormone-responsive cells.

The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter [Maniatis et al. (1987) *Science 236:*1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.]. Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host range. Examples include the SV40 early gene enhancer [Dijkema et al (1985) *EMBO J. 4*:761] and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus [Gorman et al. (1982b) *Proc. Natl. Acad. Sci. 79*:6777] and from human cytomegalovirus [Boshart et al. (1985) *Cell 41*:521]. Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion [Sassone-Corsi and Borelli (1986) *Trends Genet. 2*:215; Maniatis et al. (1987) *Science* 236:1237].

A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus triparite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation [Birnstiel et al. (1985) *Cell 41*:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot (1989) *Trends Biochem. Sci. 14*:105]. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminater/polyadenylation signals include those derived from SV40 [Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*].

Usually, the above described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 [Gluzman (1981) *Cell 23*:175] or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replicaton systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 [Kaufman et al. (1989) *Mol. Cell. Biol. 9*:946] and pHEBO [Shimizu et al. (1986) *Mol. Cell. Biol. 6*:1074].

The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*eg*. Hep G2), and a number of other cell lines.

ii. Baculovirus Systems

The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its owned set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (*eg*. plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon

will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) *17*:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, *42*:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlak et al., (1988), *J. Gen. Virol.* *69*:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, *73*:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect

origin, such as those derived from genes encoding human α-interferon, Maeda et al., (1985), *Nature 315*:592; human gastrin-releasing peptide, Lebacq-Verheyden et al., (1988), *Molec. Cell. Biol. 8*:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA, 82*:8404; mouse IL-3, (Miyajima et al., (1987) *Gene 58*:273; and human glucocerebrosidase, Martin et al. (1988) *DNA, 7*:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) *3*:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays 4*:91.The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15 μm in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative of recombinant virus) of occlusion bodies. "Current Protocols in Microbiology" Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti* , *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (WO 89/046699; Carbonell et al., (1985) *J. Virol. 56*:153; Wright (1986) *Nature 321*:718; Smith et al., (1983) *Mol. Cell. Biol. 3*:2156; and see generally, Fraser, *et al.* (1989) *In Vitro Cell. Dev. Biol. 25*:225).

~~Cells and cell culture media are commercially available for both direct and fusion expression of~~ heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. *See, eg.* Summers and Smith *supra.*

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified

by such techniques as chromatography, eg. HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, eg. proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

iii. Plant Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as: US 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., Nucleic Acids Research 15:2515-2535 (1987); Wirsel et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, Gibberellins: in: *Advanced Plant Physiology*,. Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host.

The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for Agrobacterium transformations, T DNA sequences for Agrobacterium-mediated transfer to plant chromosomes. Where the heterologous gene is not readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmink and Dons, 1993, *Plant Mol. Biol. Reptr*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested.

Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's splicosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet*, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al., *Nature*, 296, 72-74, 1982. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum,*

Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersion, Nicotiana, Solanum, Petunia, Digitalis, Majorana, Cichorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Hererocallis, Nemesia, Pelargonium, Panicum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Lolium, Zea, Triticum, Sorghum, and *Datura.*

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an

operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in Escherichia coli (E. coli) [Raibaud *et al.* (1984) *Annu. Rev. Genet. 18*:173]. Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) [Chang *et al.* (1977) *Nature 198*:1056], and maltose. Additional examples include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) [Goeddel *et al.* (1980) *Nuc. Acids Res. 8*:4057; Yelverton *et al.* (1981) *Nucl. Acids Res. 9*:731; US patent 4,738,921; EP-A-0036776 and EP-A-0121775]. The g-laotamase (*bla*) promoter system [Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)], bacteriophage lambda PL [Shimatake *et al.* (1981) *Nature 292*:128] and T5 [US patent 4,689,406] promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter [US patent 4,551,433]. For example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor [Amann *et al.* (1983) *Gene 25*:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci. 80*:21]. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system [Studier *et al.* (1986) *J. Mol. Biol. 189*:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci. 82*:1074]. In addition, a

hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO-A-0 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon [Shine *et al.* (1975) *Nature 254*:34]. The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' and of *E. coli* 16S rRNA [Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberger)]. To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site [Sambrook *et al.* (1989) "Expression of cloned genes in Escherichia coli." In *Molecular Cloning: A Laboratory Manual*].

A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* on *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO-A-0 219 237).

Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene [Nagai *et al.* (1984) *Nature 309*:810]. Fusion proteins can also be made with sequences from the *lacZ* [Jia *et al.* (1987) *Gene 60*:197], *trpE* [Allen et al. (1987) J. Biotechnol. 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol. 135*:11], and *Chey* [EP-A-0 324 647] genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*eg.*

ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated [Miller *et al.* (1989) *Bio/Technology* 7:698].

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria [US patent 4,336,336]. The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) [Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Ghrayeb *et al.* (1984) *EMBO J. 3*:2437] and the *E. coli* alkaline phosphatase signal sequence (*phoA*) [Oka *et al.* (1985) *Proc. Natl. Acad. Sci. 82*:7212]. As an additional example, the signal sequence of the alpha-amylase gene from various Bacillus strains can be used to secrete heterologous proteins from *B. subtilis* [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 244 042].

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a

prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various Bacillus strains integrate into the Bacillus chromosome (EP-A- 0 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline [Davies *et al.* (1978) *Annu. Rev. Microbiol. 32*:469]. Selectable markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable market that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: Bacillus subtilis [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541], Escherichia coli [Shimatake *et al.* (1981) *Nature 292*:128; Amann *et al.* (1985) *Gene 40*:183; Studier *et al.* (1986) *J. Mol. Biol. 189*:113; EP-A-0 036 776,EP-A-0 136 829 and EP-A-0 136 907], Streptococcus cremoris [Powell *et al.* (1988) *Appl. Environ. Microbiol. 54*:655];

Streptococcus lividans [Powell *et al.* (1988) *Appl. Environ. Microbiol. 54*:655], Streptomyces lividans [US patent 4,745,056].

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with $CaCl_2$ or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. See *eg.* [Masson *et al.* (1989) *FEMS Microbiol. Lett. 60*:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541, Bacillus], [Miller *et al.* (1988) *Proc. Natl. Acad. Sci. 85*:856; Wang *et al.* (1990) *J. Bacteriol. 172*:949, Campylobacter], [Cohen *et al.* (1973) *Proc. Natl. Acad. Sci. 69*:2110; Dower *et al.* (1988) *Nucleic Acids Res. 16*:6127; Kushner (1978) "An improved method for transformation of Escherichia coli with ColE1-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol. 53*:159; Taketo (1988) *Biochim. Biophys. Acta 949*:318; Escherichia], [Chassy *et al.* (1987) *FEMS Microbiol. Lett. 44*:173 Lactobacillus]; [Fiedler *et al.* (1988) *Anal. Biochem 170*:38, Pseudomonas]; [Augustin *et al.* (1990) *FEMS Microbiol. Lett. 66*:203, Staphylococcus], [Barany *et al.* (1980) *J. Bacteriol. 144*:698; Harlander (1987) "Transformation of Streptococcus lactis by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun. 32*:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol. 54*:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology 1*:412, Streptococcus].

v. Yeast Expression

Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EP-A-0 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO-A-0 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences [Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA 80*:1].

In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (US Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of either the *ADH2, GAL4, GAL10,* OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EP-A-0 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia,* [Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA 77*:1078; Henikoff *et al.* (1981) *Nature 283*:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol. 96*:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast Saccharomyces cerevisiae," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene 11*:163; Panthier *et al.* (1980) *Curr. Genet. 2*:109;].

A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of

heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See *eg.* EP-A-0 196 056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*eg.* ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (*eg.* WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EP-A-0 012 873; JPO. 62,096,086) and the A-factor gene (US patent 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EP-A-0 060 057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (US Patents 4,546,083 and 4,870,008; EP-A-0 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alphafactor. (*eg.* see WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the

polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 [Botstein *et al.* (1979) *Gene 8*:17-24], pCl/1 [Brake *et al.* (1984) *Proc. Natl. Acad. Sci USA 81*:4642-4646], and YRp17 [Stinchcomb *et al.* (1982) *J. Mol. Biol. 158*:157]. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Enter a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See *eg.* Brake *et al.*, *supra.*

Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome [Orr-Weaver *et al.* (1983) *Methods in Enzymol. 101*:228-245]. An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al.*, *supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced [Rine *et al.* (1983) *Proc. Natl. Acad. Sci. USA 80*:6750]. The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers

may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions [Butt *et al.* (1987) *Microbiol, Rev. 51*:351].

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors have been developed for, *inter alia*, the following yeasts:Candida albicans [Kurtz, *et al.* (1986) *Mol. Cell. Biol. 6*:142], Candida maltosa [Kunze, *et al.* (1985) *J. Basic Microbiol. 25*:141]. Hansenula polymorpha [Gleeson, *et al.* (1986) *J. Gen. Microbiol. 132*:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet. 202*:302], Kluyveromyces fragilis [Das, *et al.* (1984) *J. Bacteriol. 158*:1165], Kluyveromyces lactis [De Louvencourt *et al.* (1983) *J. Bacteriol. 154*:737; Van den Berg *et al.* (1990) *Bio/Technology 8*:135], Pichia guillerimondii [Kunze *et al.* (1985) *J. Basic Microbiol. 25*:141], Pichia pastoris [Cregg, *et al.* (1985) *Mol. Cell. Biol. 5*:3376; US Patent Nos. 4,837,148 and 4,929,555], Saccharomyces cerevisiae [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA 75*:1929; Ito *et al.* (1983) *J. Bacteriol. 153*:163], Schizosaccharomyces pombe [Beach and Nurse (1981) *Nature 300*:706], and Yarrowia lipolytica [Davidow, *et al.* (1985) *Curr. Genet. 10*:380471 Gaillardin, *et al.* (1985) *Curr. Genet. 10*:49].

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See *eg.* [Kurtz *et al.* (1986) *Mol. Cell. Biol. 6*:142; Kunze *et al.* (1985) *J. Basic Microbiol. 25*:141; Candida]; [Gleeson *et al.* (1986) *J. Gen. Microbiol. 132*:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet. 202*:302; Hansenula]; [Das *et al.* (1984) *J. Bacteriol. 158*:1165; De Louvencourt *et al.* (1983) *J. Bacteriol. 154*:1165; Van den Berg *et al.* (1990) *Bio/Technology 8*:135; Kluyveromyces]; [Cregg *et al.* (1985) *Mol. Cell. Biol. 5*:3376; Kunze *et al.* (1985) *J. Basic Microbiol. 25*:141; US Patent Nos. 4,837,148 and 4,929,555; Pichia]; [Hinnen *et al.* (1978) *Proc.*

Natl. Acad. Sci. USA 75;1929; Ito *et al.* (1983) *J. Bacteriol. 153*:163 Saccharomyces]; [Beach and Nurse (1981) *Nature 300*:706; Schizosaccharomyces]; [Davidow *et al.* (1985) *Curr. Genet. 10*:39; Gaillardin *et al.* (1985) *Curr. Genet. 10*:49; Yarrowia].

Antibodies

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An "antibody combining site" is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. "Antibody" includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, humanised antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies.

Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying meningococcal proteins.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 μg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by in vitro immunization using methods known in the art, which for the purposes of this invention is considered equivalent to *in vivo* immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (*eg.* 1,000*g* for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the standard method of Kohler & Milstein [*Nature* (1975) 256:495-96], or a modification thereof. Typically, a mouse or rat is immunized as

described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells expressing membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (*eg.* hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the immunizing antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (*eg.* in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ^{32}P and ^{125}I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various labels into distinct classes, as the same label may serve in several different modes. For example, ^{125}I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with ^{125}I, or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the invention.

Pharmaceutical Compositions

Pharmaceutical compositions can comprise either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgement of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*eg.* see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Vaccines

Vaccines according to the invention may either be prophylactic (*ie.* to prevent infection) or therapeutic (*ie.* to treat disease after infection).

Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, *etc.* pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (WO 90/14837; Chapter 10 in *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (3) saponin adjuvants, such as Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (*eg.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*eg.* gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; and (6) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59™ are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), *etc.*

The immunogenic compositions (*eg.* the immunising antigen/immunogen/polypeptide/protein/nucleic acid, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

Typically, the immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of the antigenic or immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated (*eg.* nonhuman primate, primate, *etc.*), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

The immunogenic compositions are conventionally administered parenterally, *eg.* by injection, either subcutaneously, intramuscularly, or transdermally/transcutaneously (*eg.* WO98/20734). Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed [*eg.* Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648; see later herein].

Gene Delivery Vehicles

Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence in vivo can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art and we contemplate that any retroviral gene therapy vector is employable in the invention, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses *eg.* MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (*eg.* HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J*

Virol 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC Nol VR-590), Kirsten, Harvey Sarcoma Virus and Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698, WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive

nucleotides in each AAV inverted terminal repeat (*ie.* there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. ~~Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus~~ (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in US Serial No. 08/405,627, filed March 15, 1995,WO94/21792, WO92/10578, WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, Nature 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example ATCC VR-925; Triniti virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic

acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No.08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials, hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem.* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in US 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or

ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for example, use of hand-held gene transfer particle gun, as described in US 5,149,655; use of ionizing radiation for activating transferred gene, as described in US 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, Biochemistry, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprises therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for ~~expression of recombinant proteins. The subjects to be treated can be mammals or birds. Also,~~ human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*eg.* see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in *eg.* WO93/14778. Examples of cells useful in ex vivo applications include, for example, stem cells, particularly hematopoetic, lymph cells, macrophages, dendritic cells, or tumor cells..

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

Polynucleotide and polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

A.Polypeptides

One example are polypeptides which include, without limitation: asioloorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of plasmodium falciparum known as RII.

B.Hormones, Vitamins, *etc.*

Other groups that can be included are, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C.Polyalkylenes, Polysaccharides, *etc.*

Also, polyalkylene glycol can be included with the desired polynucleotides/polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethlylene glycol. In addition, mono-, di-,

or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide)

D.Lipids, and Liposomes

The desired polynucleotide/polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boerhinger). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, *eg.* Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting

materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammelar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See *eg.* Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77); Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348); Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

E.Lipoproteins

In addition, lipoproteins can be included with the polynucleotide/polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E, over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

The amino acid of these apoproteins are known and are described in, for example, Breslow (1985) Annu Rev. Biochem 54:699; Law (1986) Adv. Exp Med. Biol. 151:162; Chen (1986) J Biol Chem

261:12918; Kane (1980) Proc Natl Acad Sci USA 77:2465; and Utermann (1984) Hum Genet 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol. (supra)*; Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750. Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443. Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Techniologies, Inc., Stoughton, Massachusetts, USA. Further description of lipoproteins can be found in Zuckermann *et al.* PCT/US97/14465.

F.Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide/polypeptide to be delivered.

~~Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are~~ capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both in vitro, ex vivo, and in vivo applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as (X174, transcriptional factors also contain domains that bind DNA and

therefore may be useful as nucleic aid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and purtrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

Synthetic polycationic agents which are useful include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides/polypeptides.

Immunodiagnostic Assays

Meningogoccal antigens of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-meningococcal antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to meningococcal proteins within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, *etc.*) required for the conduct of the assay, as well as suitable set of assay instructions.

Nucleic Acid Hybridisation

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the stringency of the washing conditions following hybridization. See Sambrook *et al.* [*supra*] Volume 2, chapter 9, pages 9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated Tm of the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1μg for a plasmid or phage digest to 10^{-9} to 10^{-8} g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 μg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of 10^8 cpm/μg. For a single-copy mammalian gene a conservative approach would start with 10 μg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than 10^8 cpm/μg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature (Tm) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$Tm = 81 + 16.6(\log_{10}Ci) + 0.4[\%(G + C)] - 0.6(\%formamide) - 600/n - 1.5(\%mismatch).$$

where Ci is the salt concentration (monovalent ions) and *n* is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138: 267-284).

In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (*ie.* stringency), it becomes less likely for hybridization to occur between strands that are nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

Nucleic Acid Probe Assays

Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to "hybridize" with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

The nucleic acid probes will hybridize to the meningococcal nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native meningococcal sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to mRNA, and so a cDNA probe should be complementary to the non-coding sequence.

The probe sequence need not be identical to the meningococcal sequence (or its complement) — some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid probe can include additional nucleotides to stabilize the formed duplex. Additional meningococcal sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a meningococcal sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a meningococcal sequence in order to hybridize therewith and thereby form a duplex which can be detected.

The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably at least 30 nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* [*J. Am. Chem. Soc.* (1981) 103:3185], or according to Urdea *et al.* [*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461], or using commercially available automated oligonucleotide synthesizers.

The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated *eg.* backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase *in vivo* half-life, alter RNA affinity, increase nuclease resistance *etc.* [*eg.* see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387]; analogues such as peptide nucleic acids may also be used [*eg.* see Corey (1997) *TIBTECH* 15:224-229; Buchardt *et al.* (1993) *TIBTECH* 11:384-386].

Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis *et al.* [*Meth. Enzymol.* (1987) 155: 335-350]; US patents 4,683,195 and 4,683,202. Two "primer" nucleotides hybridize with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its complement) to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired meningococcal sequence.

A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labelled probe will hybridize to the meningococcal sequence (or its complement).

Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al* [*supra*]. mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labelled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labelled with a radioactive moiety.

EXAMPLES OF PREFERRED FRAGMENTS

The protein sequences disclosed in PCT/IB99/00103 have been subjected to computer analysis to predict antigenic peptide fragments within the full-length proteins. Three algorithms have been used in this analysis:

- **AMPHI** This program has been used to predict T-cell epitopes [Gao *et al.* (1989) *J. Immunol.* 143:3007; Roberts *et al.* (1996) *AIDS Res Hum Retrovir* 12:593; Quakyi *et al.* (1992) *Scand J Immunol* suppl.11:9] and is available in the Protean package of DNASTAR, Inc. (1228 South Park Street, Madison, Wisconsin 53715 USA).

- **ANTIGENIC INDEX** as disclosed by Jameson & Wolf (1988) The antigenic index: a novel algorithm for predicting antigenic determinants. CABIOS, 4:181:186

- **HYDROPHILICITY** as disclosed by Hopp & Woods (1981) Prediction of protein antigenic determinants from amino acid sequences. PNAS, 78:3824-3828

Table I indicates preferred fragments of the proteins disclosed in Annex I. The three algorithms often identify the same fragments (*eg.* ORF100 – the fragment from residue 98 to residue 109, and the fragments from residue 111 to residue 121). Such multiply-identified fragments are particularly preferred. The algorithms often identify overlapping fragments (*eg.* ORF100 – AMPHI identifies residues 143-152, and Antigenic Index identified residues 148-157). The invention explicitly includes fragments resulting from a combination of these overlapping fragments (*eg.* the fragment from residue 143 to residue 157, in the case of ORF100). Fragments separated by a single amino acid are also often identified (*eg.* ORF48-1 hydrophilicity 334-342 and 344-349). The invention also includes fragments spanning the two extremes of such "adjacent" fragments (*eg.* 334-349 for ORF48-1).

TABLE I – 1769 fragments of the proteins disclosed in Annex I.

Key:

– SEQ ID 1 of the present application is amino acids 6 to 14 of ORF38-1 disclosed in Annex I, *etc.*

SEQ ID	ORF (Annex I)	Algorithm	Amino acids
1.	38-1	AMPHI	6-14
2.	38-1	AMPHI	57-59
3.	38-1	AMPHI	67-76

4.	38-1	AMPHI	92-100
5.	38-1	AMPHI	127-137
6.	38-1	AMPHI	149-166
7.	38-1	AMPHI	210-215
8.	38-1	AMPHI	231-236
9.	38-1	AMPHI	270-272
10.	38-1	AMPHI	303-320
11.	38-1	Antigenic Index	16-34
12.	38-1	Antigenic Index	37-42
13.	38-1	Antigenic Index	46-64
14.	38-1	Antigenic Index	72-91
15.	38-1	Antigenic Index	94-112
16.	38-1	Antigenic Index	114-117
17.	38-1	Antigenic Index	124-136
18.	38-1	Antigenic Index	143-146
19.	38-1	Antigenic Index	148-160
20.	38-1	Antigenic Index	167-195
21.	38-1	Antigenic Index	201-216
22.	38-1	Antigenic Index	218-240
23.	38-1	Antigenic Index	244-252
24.	38-1	Antigenic Index	257-278
25.	38-1	Antigenic Index	282-290
26.	38-1	Antigenic Index	308-314
27.	38-1	Hydrophilicity	21-34
28.	38-1	Hydrophilicity	37-42
29.	38-1	Hydrophilicity	47-55
30.	38-1	Hydrophilicity	57-61
31.	38-1	Hydrophilicity	72-74
32.	38-1	Hydrophilicity	76-78
33.	38-1	Hydrophilicity	82-91
34.	38-1	Hydrophilicity	94-101
35.	38-1	Hydrophilicity	108-112
36.	38-1	Hydrophilicity	126-136
37.	38-1	Hydrophilicity	143-146
38.	38-1	Hydrophilicity	148-160
39.	38-1	Hydrophilicity	167-195
40.	38-1	Hydrophilicity	221-223
41.	38-1	Hydrophilicity	226-236
42.	38-1	Hydrophilicity	244-250
43.	38-1	Hydrophilicity	257-274

44.	38-1	Hydrophilicity	282-286
45.	38-1	Hydrophilicity	311-314
46.	38a	AMPHI	6-14
47.	38a	AMPHI	57-59
48.	38a	AMPHI	67-76
49.	38a	AMPHI	92-100
50.	38a	AMPHI	127-137
51.	38a	AMPHI	149-166
52.	38a	AMPHI	210-215
53.	38a	AMPHI	223-225
54.	38a	AMPHI	231-236
55.	38a	AMPHI	270-272
56.	38a	AMPHI	303-320
57.	38a	Antigenic Index	16-34
58.	38a	Antigenic Index	37-42
59.	38a	Antigenic Index	46-64
60.	38a	Antigenic Index	72-91
61.	38a	Antigenic Index	94-112
62.	38a	Antigenic Index	114-117
63.	38a	Antigenic Index	124-136
64.	38a	Antigenic Index	143-146
65.	38a	Antigenic Index	148-160
66.	38a	Antigenic Index	165-195
67.	38a	Antigenic Index	201-216
68.	38a	Antigenic Index	218-240
69.	38a	Antigenic Index	244-252
70.	38a	Antigenic Index	257-278
71.	38a	Antigenic Index	282-290
72.	38a	Antigenic Index	308-314
73.	38a	Hydrophilicity	21-34
74.	38a	Hydrophilicity	37-42
75.	38a	Hydrophilicity	47-55
76.	38a	Hydrophilicity	57-61
77.	38a	Hydrophilicity	72-74
78.	38a	Hydrophilicity	76-78
79.	38a	Hydrophilicity	82-91
80.	38a	Hydrophilicity	94-101
81.	38a	Hydrophilicity	108-112
82.	38a	Hydrophilicity	126-136
83.	38a	Hydrophilicity	143-146

84.	38a	Hydrophilicity	148-160
85.	38a	Hydrophilicity	165-195
86.	38a	Hydrophilicity	221-223
87.	38a	Hydrophilicity	226-236
88.	38a	Hydrophilicity	244-250
89.	38a	Hydrophilicity	257-273
90.	38a	Hydrophilicity	282-286
91.	38a	Hydrophilicity	311-314
92.	39-1	AMPHI	6-13
93.	39-1	AMPHI	21-24
94.	39-1	AMPHI	37-40
95.	39-1	AMPHI	60-75
96.	39-1	AMPHI	118-122
97.	39-1	AMPHI	134-139
98.	39-1	AMPHI	165-183
99.	39-1	AMPHI	192-195
100.	39-1	AMPHI	233-241
101.	39-1	AMPHI	247-267
102.	39-1	AMPHI	273-275
103.	39-1	AMPHI	299-308
104.	39-1	AMPHI	310-319
105.	39-1	AMPHI	322-330
106.	39-1	AMPHI	338-347
107.	39-1	AMPHI	358-364
108.	39-1	AMPHI	366-368
109.	39-1	AMPHI	376-378
110.	39-1	AMPHI	385-392
111.	39-1	AMPHI	413-416
112.	39-1	AMPHI	421-424
113.	39-1	AMPHI	429-438
114.	39-1	AMPHI	445-454
115.	39-1	AMPHI	456-458
116.	39-1	AMPHI	498-500
117.	39-1	AMPHI	512-519
118.	39-1	AMPHI	576-587
119.	39-1	AMPHI	589-600
120.	39-1	AMPHI	650-652
121.	39-1	AMPHI	670-674
122.	39-1	Antigenic Index	26-32
123.	39-1	Antigenic Index	35-45

124.	39-1	Antigenic Index	54-69
125.	39-1	Antigenic Index	79-84
126.	39-1	Antigenic Index	88-96
127.	39-1	Antigenic Index	105-110
128.	39-1	Antigenic Index	117-124
129.	39-1	Antigenic Index	152-154
130.	39-1	Antigenic Index	190-192
131.	39-1	Antigenic Index	222-231
132.	39-1	Antigenic Index	246-265
133.	39-1	Antigenic Index	292-295
134.	39-1	Antigenic Index	318-335
135.	39-1	Antigenic Index	353-362
136.	39-1	Antigenic Index	370-372
137.	39-1	Antigenic Index	402-404
138.	39-1	Antigenic Index	406-408
139.	39-1	Antigenic Index	419-421
140.	39-1	Antigenic Index	446-449
141.	39-1	Antigenic Index	453-460
142.	39-1	Antigenic Index	465-469
143.	39-1	Antigenic Index	476-487
144.	39-1	Antigenic Index	491-499
145.	39-1	Antigenic Index	505-514
146.	39-1	Antigenic Index	522-536
147.	39-1	Antigenic Index	557-567
148.	39-1	Antigenic Index	569-575
149.	39-1	Antigenic Index	577-580
150.	39-1	Antigenic Index	593-599
151.	39-1	Antigenic Index	603-619
152.	39-1	Antigenic Index	626-628
153.	39-1	Antigenic Index	634-637
154.	39-1	Antigenic Index	639-647
155.	39-1	Antigenic Index	655-658
156.	39-1	Antigenic Index	672-674
157.	39-1	Antigenic Index	677-686
158.	39-1	Antigenic Index	688-691
159.	39-1	Antigenic Index	693-699
160.	39-1	Antigenic Index	707-710
161.	39-1	Hydrophilicity	28-32
162.	39-1	Hydrophilicity	38-44
163.	39-1	Hydrophilicity	54-69

164.	39-1	Hydrophilicity	80-83
165.	39-1	Hydrophilicity	89-96
166.	39-1	Hydrophilicity	117-119
167.	39-1	Hydrophilicity	121-123
168.	39-1	Hydrophilicity	152-154
169.	39-1	Hydrophilicity	224-231
170.	39-1	Hydrophilicity	247-265
171.	39-1	Hydrophilicity	318-332
172.	39-1	Hydrophilicity	357-361
173.	39-1	Hydrophilicity	402-404
174.	39-1	Hydrophilicity	406-408
175.	39-1	Hydrophilicity	446-449
176.	39-1	Hydrophilicity	454-459
177.	39-1	Hydrophilicity	465-469
178.	39-1	Hydrophilicity	476-487
179.	39-1	Hydrophilicity	491-499
180.	39-1	Hydrophilicity	506-514
181.	39-1	Hydrophilicity	525-535
182.	39-1	Hydrophilicity	560-567
183.	39-1	Hydrophilicity	573-575
184.	39-1	Hydrophilicity	577-580
185.	39-1	Hydrophilicity	594-596
186.	39-1	Hydrophilicity	605-607
187.	39-1	Hydrophilicity	611-619
188.	39-1	Hydrophilicity	634-637
189.	39-1	Hydrophilicity	639-647
190.	39-1	Hydrophilicity	672-674
191.	39-1	Hydrophilicity	677-686
192.	39-1	Hydrophilicity	688-690
193.	39-1	Hydrophilicity	693-695
194.	39a	AMPHI	6-13
195.	39a	AMPHI	21-24
196.	39a	AMPHI	37-40
197.	39a	AMPHI	60-75
198.	39a	AMPHI	118-122
199.	39a	AMPHI	134-139
200.	39a	AMPHI	165-183
201.	39a	AMPHI	192-195
202.	39a	AMPHI	233-241
203.	39a	AMPHI	247-267

204.	39a	AMPHI	273-275
205.	39a	AMPHI	299-308
206.	39a	AMPHI	310-319
207.	39a	AMPHI	322-330
208.	39a	AMPHI	338-347
209.	39a	AMPHI	358-364
210.	39a	AMPHI	366-368
211.	39a	AMPHI	376-378
212.	39a	AMPHI	385-392
213.	39a	AMPHI	413-416
214.	39a	AMPHI	421-424
215.	39a	AMPHI	429-438
216.	39a	AMPHI	445-454
217.	39a	AMPHI	456-458
218.	39a	AMPHI	498-500
219.	39a	AMPHI	512-520
220.	39a	AMPHI	576-587
221.	39a	AMPHI	589-600
222.	39a	AMPHI	650-652
223.	39a	AMPHI	670-674
224.	39a	Antigenic Index	26-32
225.	39a	Antigenic Index	35-45
226.	39a	Antigenic Index	54-69
227.	39a	Antigenic Index	79-84
228.	39a	Antigenic Index	89-96
229.	39a	Antigenic Index	103-110
230.	39a	Antigenic Index	117-124
231.	39a	Antigenic Index	152-154
232.	39a	Antigenic Index	190-192
233.	39a	Antigenic Index	222-231
234.	39a	Antigenic Index	246-265
235.	39a	Antigenic Index	292-295
236.	39a	Antigenic Index	318-335
237.	39a	Antigenic Index	353-362
238.	39a	Antigenic Index	370-372
239.	39a	Antigenic Index	402-404
240.	39a	Antigenic Index	406-408
241.	39a	Antigenic Index	419-421
242.	39a	Antigenic Index	446-449
243.	39a	Antigenic Index	453-460

244.	39a	Antigenic Index	465-469
245.	39a	Antigenic Index	476-487
246.	39a	Antigenic Index	491-499
247.	39a	Antigenic Index	505-514
248.	39a	Antigenic Index	529-535
249.	39a	Antigenic Index	557-567
250.	39a	Antigenic Index	569-575
251.	39a	Antigenic Index	577-580
252.	39a	Antigenic Index	593-599
253.	39a	Antigenic Index	603-619
254.	39a	Antigenic Index	626-628
255.	39a	Antigenic Index	634-637
256.	39a	Antigenic Index	639-647
257.	39a	Antigenic Index	655-658
258.	39a	Antigenic Index	672-674
259.	39a	Antigenic Index	677-686
260.	39a	Antigenic Index	688-691
261.	39a	Antigenic Index	693-699
262.	39a	Antigenic Index	707-710
263.	39a	Hydrophilicity	28-32
264.	39a	Hydrophilicity	38-44
265.	39a	Hydrophilicity	54-69
266.	39a	Hydrophilicity	80-83
267.	39a	Hydrophilicity	89-95
268.	39a	Hydrophilicity	105-108
269.	39a	Hydrophilicity	117-119
270.	39a	Hydrophilicity	121-123
271.	39a	Hydrophilicity	152-154
272.	39a	Hydrophilicity	224-231
273.	39a	Hydrophilicity	247-265
274.	39a	Hydrophilicity	318-332
275.	39a	Hydrophilicity	357-361
276.	39a	Hydrophilicity	402-404
277.	39a	Hydrophilicity	406-408
278.	39a	Hydrophilicity	446-449
279.	39a	Hydrophilicity	454-459
280.	39a	Hydrophilicity	465-469
281.	39a	Hydrophilicity	476-487
282.	39a	Hydrophilicity	491-499
283.	39a	Hydrophilicity	506-514

284.	39a	Hydrophilicity	529-535
285.	39a	Hydrophilicity	560-567
286.	39a	Hydrophilicity	573-575
287.	39a	Hydrophilicity	577-580
288.	39a	Hydrophilicity	594-596
289.	39a	Hydrophilicity	605-607
290.	39a	Hydrophilicity	611-619
291.	39a	Hydrophilicity	634-637
292.	39a	Hydrophilicity	639-647
293.	39a	Hydrophilicity	672-674
294.	39a	Hydrophilicity	677-686
295.	39a	Hydrophilicity	688-690
296.	39a	Hydrophilicity	693-695
297.	40-1	AMPHI	6-14
298.	40-1	AMPHI	16-19
299.	40-1	AMPHI	22-27
300.	40-1	AMPHI	30-33
301.	40-1	AMPHI	41-44
302.	40-1	AMPHI	62-68
303.	40-1	AMPHI	129-139
304.	40-1	AMPHI	161-165
305.	40-1	AMPHI	181-191
306.	40-1	AMPHI	199-202
307.	40-1	AMPHI	215-220
308.	40-1	AMPHI	237-249
309.	40-1	AMPHI	298-302
310.	40-1	AMPHI	313-318
311.	40-1	AMPHI	335-342
312.	40-1	AMPHI	376-383
313.	40-1	AMPHI	399-402
314.	40-1	AMPHI	426-428
315.	40-1	AMPHI	430-433
316.	40-1	AMPHI	435-437
317.	40-1	AMPHI	479-482
318.	40-1	AMPHI	491-511
319.	40-1	AMPHI	523-525
320.	40-1	AMPHI	560-563
321.	40-1	Antigenic Index	21-32
322.	40-1	Antigenic Index	49-61
323.	40-1	Antigenic Index	64-66

324.	40-1	Antigenic Index	74-92
325.	40-1	Antigenic Index	98-123
326.	40-1	Antigenic Index	129-135
327.	40-1	Antigenic Index	138-176
328.	40-1	Antigenic Index	193-195
329.	40-1	Antigenic Index	199-219
330.	40-1	Antigenic Index	226-240
331.	40-1	Antigenic Index	242-245
332.	40-1	Antigenic Index	251-257
333.	40-1	Antigenic Index	261-276
334.	40-1	Antigenic Index	279-306
335.	40-1	Antigenic Index	308-346
336.	40-1	Antigenic Index	352-367
337.	40-1	Antigenic Index	375-378
338.	40-1	Antigenic Index	384-406
339.	40-1	Antigenic Index	408-420
340.	40-1	Antigenic Index	423-426
341.	40-1	Antigenic Index	428-438
342.	40-1	Antigenic Index	453-459
343.	40-1	Antigenic Index	462-481
344.	40-1	Antigenic Index	485-494
345.	40-1	Antigenic Index	506-518
346.	40-1	Antigenic Index	535-539
347.	40-1	Antigenic Index	544-552
348.	40-1	Antigenic Index	559-566
349.	40-1	Antigenic Index	571-582
350.	40-1	Hydrophilicity	21-32
351.	40-1	Hydrophilicity	51-61
352.	40-1	Hydrophilicity	64-66
353.	40-1	Hydrophilicity	75-92
354.	40-1	Hydrophilicity	100-122
355.	40-1	Hydrophilicity	129-135
356.	40-1	Hydrophilicity	140-145
357.	40-1	Hydrophilicity	149-152
358.	40-1	Hydrophilicity	157-161
359.	40-1	Hydrophilicity	163-175
360.	40-1	Hydrophilicity	199-201
361.	40-1	Hydrophilicity	203-219
362.	40-1	Hydrophilicity	227-240
363.	40-1	Hydrophilicity	251-257

364.	40-1	Hydrophilicity	261-276
365.	40-1	Hydrophilicity	279-306
366.	40-1	Hydrophilicity	308-318
367.	40-1	Hydrophilicity	320-328
368.	40-1	Hydrophilicity	334-341
369.	40-1	Hydrophilicity	354-356
370.	40-1	Hydrophilicity	359-366
371.	40-1	Hydrophilicity	392-398
372.	40-1	Hydrophilicity	400-405
373.	40-1	Hydrophilicity	410-420
374.	40-1	Hydrophilicity	429-438
375.	40-1	Hydrophilicity	463-467
376.	40-1	Hydrophilicity	471-480
377.	40-1	Hydrophilicity	487-493
378.	40-1	Hydrophilicity	506-518
379.	40-1	Hydrophilicity	547-552
380.	40-1	Hydrophilicity	575-579
381.	40a	AMPHI	6-10
382.	40a	AMPHI	19-27
383.	40a	AMPHI	30-33
384.	40a	AMPHI	41-44
385.	40a	AMPHI	61-72
386.	40a	AMPHI	78-81
387.	40a	AMPHI	92-94
388.	40a	AMPHI	128-130
389.	40a	AMPHI	132-134
390.	40a	AMPHI	161-165
391.	40a	AMPHI	181-193
392.	40a	AMPHI	197-199
393.	40a	AMPHI	204-211
394.	40a	AMPHI	213-218
395.	40a	AMPHI	227-229
396.	40a	AMPHI	237-249
397.	40a	AMPHI	298-302
398.	40a	AMPHI	313-318
399.	40a	AMPHI	335-342
400.	40a	AMPHI	376-383
401.	40a	AMPHI	399-402
402.	40a	AMPHI	426-428
403.	40a	AMPHI	435-437

404.	40a	AMPHI	475-483
405.	40a	AMPHI	492-512
406.	40a	AMPHI	524-526
407.	40a	AMPHI	561-564
408.	40a	Antigenic Index	21-34
409.	40a	Antigenic Index	50-64
410.	40a	Antigenic Index	75-83
411.	40a	Antigenic Index	88-97
412.	40a	Antigenic Index	105-122
413.	40a	Antigenic Index	129-134
414.	40a	Antigenic Index	140-176
415.	40a	Antigenic Index	190-207
416.	40a	Antigenic Index	211-217
417.	40a	Antigenic Index	224-240
418.	40a	Antigenic Index	242-245
419.	40a	Antigenic Index	250-255
420.	40a	Antigenic Index	260-276
421.	40a	Antigenic Index	279-306
422.	40a	Antigenic Index	308-346
423.	40a	Antigenic Index	352-367
424.	40a	Antigenic Index	375-378
425.	40a	Antigenic Index	384-406
426.	40a	Antigenic Index	408-420
427.	40a	Antigenic Index	423-438
428.	40a	Antigenic Index	453-468
429.	40a	Antigenic Index	471-481
430.	40a	Antigenic Index	487-493
431.	40a	Antigenic Index	507-519
~~432.~~	~~40a~~	~~Antigenic Index~~	~~536-540~~
433.	40a	Antigenic Index	545-553
434.	40a	Antigenic Index	560-567
435.	40a	Antigenic Index	572-583
436.	40a	Hydrophilicity	21-34
437.	40a	Hydrophilicity	50-64
438.	40a	Hydrophilicity	75-83
439.	40a	Hydrophilicity	88-96
440.	40a	Hydrophilicity	105-121
441.	40a	Hydrophilicity	129-134
442.	40a	Hydrophilicity	140-145
443.	40a	Hydrophilicity	148-155

444.	40a	Hydrophilicity	157-161
445.	40a	Hydrophilicity	163-175
446.	40a	Hydrophilicity	196-202
447.	40a	Hydrophilicity	211-217
448.	40a	Hydrophilicity	225-230
449.	40a	Hydrophilicity	232-240
450.	40a	Hydrophilicity	253-255
451.	40a	Hydrophilicity	261-276
452.	40a	Hydrophilicity	279-306
453.	40a	Hydrophilicity	308-318
454.	40a	Hydrophilicity	320-328
455.	40a	Hydrophilicity	334-341
456.	40a	Hydrophilicity	354-356
457.	40a	Hydrophilicity	359-366
458.	40a	Hydrophilicity	392-398
459.	40a	Hydrophilicity	400-405
460.	40a	Hydrophilicity	410-420
461.	40a	Hydrophilicity	428-438
462.	40a	Hydrophilicity	462-468
463.	40a	Hydrophilicity	472-481
464.	40a	Hydrophilicity	489-493
465.	40a	Hydrophilicity	507-519
466.	40a	Hydrophilicity	548-553
467.	40a	Hydrophilicity	576-580
468.	41-1	AMPHI	30-36
469.	41-1	AMPHI	93-98
470.	41-1	AMPHI	111-122
471.	41-1	AMPHI	126-129
472.	41-1	AMPHI	136-143
473.	41-1	AMPHI	145-150
474.	41-1	AMPHI	156-158
475.	41-1	AMPHI	186-195
476.	41-1	AMPHI	201-208
477.	41-1	AMPHI	213-223
478.	41-1	AMPHI	236-247
479.	41-1	AMPHI	250-255
480.	41-1	AMPHI	273-282
481.	41-1	AMPHI	303-309
482.	41-1	AMPHI	311-314
483.	41-1	AMPHI	329-338

484.	41-1	AMPHI	344-362
485.	41-1	AMPHI	372-377
486.	41-1	AMPHI	385-392
487.	41-1	AMPHI	409-412
488.	41-1	AMPHI	419-426
489.	41-1	AMPHI	458-463
490.	41-1	AMPHI	470-474
491.	41-1	AMPHI	486-489
492.	41-1	AMPHI	512-518
493.	41-1	AMPHI	527-551
494.	41-1	AMPHI	564-579
495.	41-1	AMPHI	593-597
496.	41-1	Antigenic Index	13-22
497.	41-1	Antigenic Index	30-38
498.	41-1	Antigenic Index	43-55
499.	41-1	Antigenic Index	73-75
500.	41-1	Antigenic Index	87-89
501.	41-1	Antigenic Index	105-112
502.	41-1	Antigenic Index	114-124
503.	41-1	Antigenic Index	136-141
504.	41-1	Antigenic Index	147-153
505.	41-1	Antigenic Index	163-166
506.	41-1	Antigenic Index	174-184
507.	41-1	Antigenic Index	195-207
508.	41-1	Antigenic Index	226-236
509.	41-1	Antigenic Index	244-246
510.	41-1	Antigenic Index	249-265
511.	41-1	Antigenic Index	281-287
512.	41-1	Antigenic Index	294-313
513.	41-1	Antigenic Index	317-342
514.	41-1	Antigenic Index	350-375
515.	41-1	Antigenic Index	379-386
516.	41-1	Antigenic Index	390-396
517.	41-1	Antigenic Index	413-422
518.	41-1	Antigenic Index	425-430
519.	41-1	Antigenic Index	436-440
520.	41-1	Antigenic Index	446-465
521.	41-1	Antigenic Index	468-495
522.	41-1	Antigenic Index	498-518
523.	41-1	Antigenic Index	520-522

524.	41-1	Antigenic Index	525-542
525.	41-1	Antigenic Index	547-558
526.	41-1	Antigenic Index	565-590
527.	41-1	Antigenic Index	595-602
528.	41-1	Antigenic Index	608-619
529.	41-1	Hydrophilicity	14-21
530.	41-1	Hydrophilicity	30-33
531.	41-1	Hydrophilicity	45-55
532.	41-1	Hydrophilicity	87-89
533.	41-1	Hydrophilicity	106-111
534.	41-1	Hydrophilicity	114-120
535.	41-1	Hydrophilicity	122-124
536.	41-1	Hydrophilicity	136-141
537.	41-1	Hydrophilicity	148-150
538.	41-1	Hydrophilicity	177-184
539.	41-1	Hydrophilicity	195-207
540.	41-1	Hydrophilicity	226-234
541.	41-1	Hydrophilicity	249-265
542.	41-1	Hydrophilicity	285-287
543.	41-1	Hydrophilicity	294-297
544.	41-1	Hydrophilicity	299-313
545.	41-1	Hydrophilicity	317-321
546.	41-1	Hydrophilicity	323-342
547.	41-1	Hydrophilicity	350-371
548.	41-1	Hydrophilicity	379-386
549.	41-1	Hydrophilicity	417-422
550.	41-1	Hydrophilicity	425-427
551.	41-1	Hydrophilicity	447-449
552.	41-1	Hydrophilicity	459-462
553.	41-1	Hydrophilicity	468-475
554.	41-1	Hydrophilicity	479-482
555.	41-1	Hydrophilicity	484-491
556.	41-1	Hydrophilicity	499-518
557.	41-1	Hydrophilicity	520-522
558.	41-1	Hydrophilicity	526-542
559.	41-1	Hydrophilicity	550-558
560.	41-1	Hydrophilicity	568-590
561.	41-1	Hydrophilicity	595-598
562.	41-1	Hydrophilicity	617-619
563.	41a	AMPHI	6-12

564.	41a	AMPHI	32-34
565.	41a	AMPHI	69-74
566.	41a	AMPHI	86-98
567.	41a	AMPHI	111-119
568.	41a	AMPHI	121-126
569.	41a	AMPHI	132-134
570.	41a	AMPHI	155-160
571.	41a	AMPHI	162-171
572.	41a	AMPHI	177-184
573.	41a	AMPHI	189-199
574.	41a	AMPHI	212-223
575.	41a	AMPHI	226-231
576.	41a	AMPHI	249-258
577.	41a	AMPHI	287-290
578.	41a	AMPHI	305-314
579.	41a	AMPHI	320-338
580.	41a	AMPHI	348-353
581.	41a	AMPHI	361-368
582.	41a	AMPHI	385-388
583.	41a	AMPHI	395-402
584.	41a	AMPHI	434-439
585.	41a	AMPHI	446-450
586.	41a	AMPHI	462-467
587.	41a	AMPHI	470-475
588.	41a	AMPHI	488-494
589.	41a	AMPHI	503-525
590.	41a	AMPHI	540-555
591.	41a	AMPHI	569-573
~~592.~~	~~41a~~	~~AMPHI~~	~~578-594~~
593.	41a	Antigenic Index	10-13
594.	41a	Antigenic Index	19-31
595.	41a	Antigenic Index	48-50
596.	41a	Antigenic Index	63-65
597.	41a	Antigenic Index	82-101
598.	41a	Antigenic Index	112-117
599.	41a	Antigenic Index	123-129
600.	41a	Antigenic Index	139-142
601.	41a	Antigenic Index	150-160
602.	41a	Antigenic Index	171-183
603.	41a	Antigenic Index	202-212

604.	41a	Antigenic Index	220-222
605.	41a	Antigenic Index	225-241
606.	41a	Antigenic Index	257-263
607.	41a	Antigenic Index	270-289
608.	41a	Antigenic Index	293-318
609.	41a	Antigenic Index	326-351
610.	41a	Antigenic Index	355-362
611.	41a	Antigenic Index	366-372
612.	41a	Antigenic Index	389-398
613.	41a	Antigenic Index	401-406
614.	41a	Antigenic Index	412-416
615.	41a	Antigenic Index	422-441
616.	41a	Antigenic Index	444-446
617.	41a	Antigenic Index	451-471
618.	41a	Antigenic Index	475-494
619.	41a	Antigenic Index	496-498
620.	41a	Antigenic Index	501-518
621.	41a	Antigenic Index	523-534
622.	41a	Antigenic Index	540-566
623.	41a	Antigenic Index	571-578
624.	41a	Antigenic Index	582-595
625.	41a	Hydrophilicity	21-31
626.	41a	Hydrophilicity	63-65
627.	41a	Hydrophilicity	83-96
628.	41a	Hydrophilicity	98-100
629.	41a	Hydrophilicity	112-117
630.	41a	Hydrophilicity	124-126
631.	41a	Hydrophilicity	153-160
~~632.~~	~~41a~~	~~Hydrophilicity~~	~~171-183~~
633.	41a	Hydrophilicity	202-210
634.	41a	Hydrophilicity	220-222
635.	41a	Hydrophilicity	225-241
636.	41a	Hydrophilicity	261-263
637.	41a	Hydrophilicity	270-273
638.	41a	Hydrophilicity	275-289
639.	41a	Hydrophilicity	293-297
640.	41a	Hydrophilicity	299-318
641.	41a	Hydrophilicity	326-347
642.	41a	Hydrophilicity	355-362
643.	41a	Hydrophilicity	393-398

644.	41a	Hydrophilicity	401-403
645.	41a	Hydrophilicity	423-425
646.	41a	Hydrophilicity	435-438
647.	41a	Hydrophilicity	454-458
648.	41a	Hydrophilicity	460-471
649.	41a	Hydrophilicity	475-494
650.	41a	Hydrophilicity	496-498
651.	41a	Hydrophilicity	502-518
652.	41a	Hydrophilicity	527-534
653.	41a	Hydrophilicity	544-566
654.	41a	Hydrophilicity	571-574
655.	41a	Hydrophilicity	593-595
656.	44-1	AMPHI	57-60
657.	44-1	AMPHI	76-79
658.	44-1	Antigenic Index	22-34
659.	44-1	Antigenic Index	38-46
660.	44-1	Antigenic Index	50-55
661.	44-1	Antigenic Index	64-70
662.	44-1	Antigenic Index	72-80
663.	44-1	Antigenic Index	83-89
664.	44-1	Antigenic Index	96-106
665.	44-1	Antigenic Index	110-124
666.	44-1	Hydrophilicity	22-34
667.	44-1	Hydrophilicity	40-46
668.	44-1	Hydrophilicity	64-69
669.	44-1	Hydrophilicity	73-80
670.	44-1	Hydrophilicity	84-89
671.	44-1	Hydrophilicity	97-106
~~672.~~	~~44-1~~	~~Hydrophilicity~~	~~120-124~~
673.	44a	AMPHI	57-60
674.	44a	AMPHI	76-79
675.	44a	Antigenic Index	23-34
676.	44a	Antigenic Index	38-46
677.	44a	Antigenic Index	50-55
678.	44a	Antigenic Index	64-70
679.	44a	Antigenic Index	72-80
680.	44a	Antigenic Index	83-89
681.	44a	Antigenic Index	96-106
682.	44a	Antigenic Index	110-124
683.	44a	Hydrophilicity	28-34

684.	44a	Hydrophilicity	40-46
685.	44a	Hydrophilicity	64-69
686.	44a	Hydrophilicity	73-80
687.	44a	Hydrophilicity	84-89
688.	44a	Hydrophilicity	97-106
689.	44a	Hydrophilicity	120-124
690.	49-1	AMPHI	16-21
691.	49-1	AMPHI	44-48
692.	49-1	AMPHI	56-61
693.	49-1	AMPHI	92-97
694.	49-1	AMPHI	118-127
695.	49-1	AMPHI	130-149
696.	49-1	AMPHI	156-178
697.	49-1	AMPHI	235-240
698.	49-1	AMPHI	253-264
699.	49-1	AMPHI	268-271
700.	49-1	AMPHI	278-285
701.	49-1	AMPHI	287-292
702.	49-1	AMPHI	298-300
703.	49-1	AMPHI	328-337
704.	49-1	AMPHI	343-350
705.	49-1	AMPHI	355-365
706.	49-1	AMPHI	378-389
707.	49-1	AMPHI	422-424
708.	49-1	AMPHI	442-450
709.	49-1	AMPHI	464-481
710.	49-1	AMPHI	486-496
711.	49-1	AMPHI	514-521
712.	49-1	AMPHI	548-551
713.	49-1	AMPHI	553-557
714.	49-1	AMPHI	562-568
715.	49-1	AMPHI	573-575
716.	49-1	AMPHI	588-590
717.	49-1	AMPHI	603-605
718.	49-1	AMPHI	614-618
719.	49-1	Antigenic Index	15-21
720.	49-1	Antigenic Index	26-43
721.	49-1	Antigenic Index	50-59
722.	49-1	Antigenic Index	61-75
723.	49-1	Antigenic Index	79-87

724.	49-1	Antigenic Index	98-108
725.	49-1	Antigenic Index	110-120
726.	49-1	Antigenic Index	122-139
727.	49-1	Antigenic Index	147-164
728.	49-1	Antigenic Index	171-179
729.	49-1	Antigenic Index	185-197
730.	49-1	Antigenic Index	214-216
731.	49-1	Antigenic Index	229-231
732.	49-1	Antigenic Index	248-266
733.	49-1	Antigenic Index	278-283
734.	49-1	Antigenic Index	289-295
735.	49-1	Antigenic Index	316-326
736.	49-1	Antigenic Index	337-349
737.	49-1	Antigenic Index	368-378
738.	49-1	Antigenic Index	386-388
739.	49-1	Antigenic Index	390-410
740.	49-1	Antigenic Index	412-414
741.	49-1	Antigenic Index	423-429
742.	49-1	Antigenic Index	438-454
743.	49-1	Antigenic Index	462-475
744.	49-1	Antigenic Index	482-500
745.	49-1	Antigenic Index	503-509
746.	49-1	Antigenic Index	521-528
747.	49-1	Antigenic Index	540-562
748.	49-1	Antigenic Index	572-579
749.	49-1	Antigenic Index	590-606
750.	49-1	Antigenic Index	610-612
751.	49-1	Antigenic Index	617-619
~~752.~~	~~49-1~~	~~Antigenic Index~~	~~626-634~~
753.	49-1	Antigenic Index	637-640
754.	49-1	Hydrophilicity	18-21
755.	49-1	Hydrophilicity	26-29
756.	49-1	Hydrophilicity	31-43
757.	49-1	Hydrophilicity	51-57
758.	49-1	Hydrophilicity	64-68
759.	49-1	Hydrophilicity	79-87
760.	49-1	Hydrophilicity	98-107
761.	49-1	Hydrophilicity	122-125
762.	49-1	Hydrophilicity	147-164
763.	49-1	Hydrophilicity	172-175

764.	49-1	Hydrophilicity	187-197
765.	49-1	Hydrophilicity	229-231
766.	49-1	Hydrophilicity	256-262
767.	49-1	Hydrophilicity	264-266
768.	49-1	Hydrophilicity	278-283
769.	49-1	Hydrophilicity	290-292
770.	49-1	Hydrophilicity	319-326
771.	49-1	Hydrophilicity	337-349
772.	49-1	Hydrophilicity	368-376
773.	49-1	Hydrophilicity	386-388
774.	49-1	Hydrophilicity	390-410
775.	49-1	Hydrophilicity	412-414
776.	49-1	Hydrophilicity	423-429
777.	49-1	Hydrophilicity	441-451
778.	49-1	Hydrophilicity	466-472
779.	49-1	Hydrophilicity	484-490
780.	49-1	Hydrophilicity	492-494
781.	49-1	Hydrophilicity	496-498
782.	49-1	Hydrophilicity	522-528
783.	49-1	Hydrophilicity	543-562
784.	49-1	Hydrophilicity	591-606
785.	49-1	Hydrophilicity	617-619
786.	49-1	Hydrophilicity	626-632
787.	49-1	Hydrophilicity	637-640
788.	49a	AMPHI	55-61
789.	49a	AMPHI	92-97
790.	49a	AMPHI	118-127
791.	49a	AMPHI	129-135
792.	49a	AMPHI	137-145
793.	49a	AMPHI	156-178
794.	49a	AMPHI	198-200
795.	49a	AMPHI	235-240
796.	49a	AMPHI	252-264
797.	49a	AMPHI	277-285
798.	49a	AMPHI	287-292
799.	49a	AMPHI	298-300
800.	49a	AMPHI	321-326
801.	49a	AMPHI	328-337
802.	49a	AMPHI	343-350
803.	49a	AMPHI	355-365

804.	49a	AMPHI	378-389
805.	49a	AMPHI	392-397
806.	49a	AMPHI	415-424
807.	49a	AMPHI	453-456
808.	49a	AMPHI	471-480
809.	49a	AMPHI	486-504
810.	49a	AMPHI	514-519
811.	49a	AMPHI	527-534
812.	49a	AMPHI	551-554
813.	49a	AMPHI	561-568
814.	49a	AMPHI	600-605
815.	49a	AMPHI	612-616
816.	49a	AMPHI	628-633
817.	49a	AMPHI	636-641
818.	49a	AMPHI	654-660
819.	49a	AMPHI	669-691
820.	49a	AMPHI	706-721
821.	49a	AMPHI	735-739
822.	49a	AMPHI	744-760
823.	49a	Antigenic Index	4-23
824.	49a	Antigenic Index	27-43
825.	49a	Antigenic Index	51-62
826.	49a	Antigenic Index	64-68
827.	49a	Antigenic Index	72-75
828.	49a	Antigenic Index	79-87
829.	49a	Antigenic Index	98-108
830.	49a	Antigenic Index	110-120
831.	49a	Antigenic Index	124-139
832.	49a	Antigenic Index	147-164
833.	49a	Antigenic Index	176-179
834.	49a	Antigenic Index	185-197
835.	49a	Antigenic Index	214-216
836.	49a	Antigenic Index	229-231
837.	49a	Antigenic Index	248-267
838.	49a	Antigenic Index	278-283
839.	49a	Antigenic Index	289-295
840.	49a	Antigenic Index	305-308
841.	49a	Antigenic Index	316-326
842.	49a	Antigenic Index	337-349
843.	49a	Antigenic Index	368-378

844.	49a	Antigenic Index	386-388
845.	49a	Antigenic Index	391-407
846.	49a	Antigenic Index	423-429
847.	49a	Antigenic Index	436-455
848.	49a	Antigenic Index	459-484
849.	49a	Antigenic Index	492-517
850.	49a	Antigenic Index	521-528
851.	49a	Antigenic Index	532-539
852.	49a	Antigenic Index	555-564
853.	49a	Antigenic Index	567-572
854.	49a	Antigenic Index	578-582
855.	49a	Antigenic Index	588-607
856.	49a	Antigenic Index	610-612
857.	49a	Antigenic Index	617-637
858.	49a	Antigenic Index	641-660
859.	49a	Antigenic Index	662-664
860.	49a	Antigenic Index	667-684
861.	49a	Antigenic Index	689-700
862.	49a	Antigenic Index	706-732
863.	49a	Antigenic Index	737-744
864.	49a	Antigenic Index	748-761
865.	49a	Hydrophilicity	4-23
866.	49a	Hydrophilicity	31-43
867.	49a	Hydrophilicity	51-53
868.	49a	Hydrophilicity	55-57
869.	49a	Hydrophilicity	64-68
870.	49a	Hydrophilicity	79-87
871.	49a	Hydrophilicity	98-106
872.	49a	Hydrophilicity	114-120
873.	49a	Hydrophilicity	130-139
874.	49a	Hydrophilicity	147-164
875.	49a	Hydrophilicity	187-197
876.	49a	Hydrophilicity	229-231
877.	49a	Hydrophilicity	249-262
878.	49a	Hydrophilicity	264-266
879.	49a	Hydrophilicity	278-283
880.	49a	Hydrophilicity	290-292
881.	49a	Hydrophilicity	319-326
882.	49a	Hydrophilicity	337-349
883.	49a	Hydrophilicity	368-376

884.	49a	Hydrophilicity	386-388
885.	49a	Hydrophilicity	391-407
886.	49a	Hydrophilicity	427-429
887.	49a	Hydrophilicity	436-439
888.	49a	Hydrophilicity	441-455
889.	49a	Hydrophilicity	459-463
890.	49a	Hydrophilicity	465-484
891.	49a	Hydrophilicity	492-513
892.	49a	Hydrophilicity	521-528
893.	49a	Hydrophilicity	559-564
894.	49a	Hydrophilicity	567-569
895.	49a	Hydrophilicity	589-591
896.	49a	Hydrophilicity	601-604
897.	49a	Hydrophilicity	620-624
898.	49a	Hydrophilicity	626-637
899.	49a	Hydrophilicity	641-660
900.	49a	Hydrophilicity	662-664
901.	49a	Hydrophilicity	668-684
902.	49a	Hydrophilicity	693-700
903.	49a	Hydrophilicity	710-732
904.	49a	Hydrophilicity	737-740
905.	49a	Hydrophilicity	759-761
906.	51-1	AMPHI	15-21
907.	51-1	AMPHI	40-54
908.	51-1	AMPHI	75-86
909.	51-1	AMPHI	108-110
910.	51-1	AMPHI	112-124
911.	51-1	AMPHI	141-148
~~912.~~	~~51-1~~	~~AMPHI~~	~~184-189~~
913.	51-1	AMPHI	211-216
914.	51-1	Antigenic Index	58-65
915.	51-1	Antigenic Index	123-127
916.	51-1	Antigenic Index	132-137
917.	51-1	Antigenic Index	149-153
918.	51-1	Antigenic Index	165-177
919.	51-1	Antigenic Index	198-204
920.	51-1	Antigenic Index	222-231
921.	51-1	Hydrophilicity	60-65
922.	51-1	Hydrophilicity	123-127
923.	51-1	Hydrophilicity	132-135

924.	51-1	Hydrophilicity	165-174
925.	51-1	Hydrophilicity	200-203
926.	51-1	Hydrophilicity	222-227
927.	51a	AMPHI	15-21
928.	51a	AMPHI	40-54
929.	51a	AMPHI	75-86
930.	51a	AMPHI	108-110
931.	51a	AMPHI	112-124
932.	51a	AMPHI	141-148
933.	51a	AMPHI	184-189
934.	51a	AMPHI	211-216
935.	51a	Hydrophilicity	60-65
936.	51a	Hydrophilicity	123-127
937.	51a	Hydrophilicity	132-135
938.	51a	Hydrophilicity	165-174
939.	51a	Hydrophilicity	200-203
940.	51a	Hydrophilicity	222-227
941.	52-1	AMPHI	48-50
942.	52-1	AMPHI	64-73
943.	52-1	Antigenic Index	19-26
944.	52-1	Antigenic Index	30-35
945.	52-1	Antigenic Index	42-52
946.	52-1	Antigenic Index	57-86
947.	52-1	Hydrophilicity	22-26
948.	52-1	Hydrophilicity	30-35
949.	52-1	Hydrophilicity	42-52
950.	52-1	Hydrophilicity	57-71
951.	52-1	Hydrophilicity	78-86
952.	69-1	AMPHI	25-27
953.	69-1	AMPHI	46-66
954.	69-1	Antigenic Index	32-41
955.	69-1	Antigenic Index	43-45
956.	69-1	Antigenic Index	71-78
957.	69-1	Hydrophilicity	32-38
958.	69-1	Hydrophilicity	71-78
959.	69a	AMPHI	25-27
960.	69a	AMPHI	46-66
961.	69a	Antigenic Index	32-41
962.	69a	Antigenic Index	43-46
963.	69a	Antigenic Index	71-78

964.	69a	Hydrophilicity	32-38
965.	69a	Hydrophilicity	71-78
966.	77-1	AMPHI	12-16
967.	77-1	AMPHI	23-33
968.	77-1	AMPHI	35-42
969.	77-1	AMPHI	51-57
970.	77-1	AMPHI	67-70
971.	77-1	AMPHI	73-79
972.	77-1	AMPHI	122-124
973.	77-1	AMPHI	130-134
974.	77-1	AMPHI	165-178
975.	77-1	AMPHI	191-211
976.	77-1	Antigenic Index	22-31
977.	77-1	Antigenic Index	34-44
978.	77-1	Antigenic Index	80-94
979.	77-1	Antigenic Index	101-104
980.	77-1	Antigenic Index	155-158
981.	77-1	Antigenic Index	167-181
982.	77-1	Hydrophilicity	22-28
983.	77-1	Hydrophilicity	38-44
984.	77-1	Hydrophilicity	80-92
985.	77-1	Hydrophilicity	171-178
986.	77a	AMPHI	8-15
987.	77a	AMPHI	24-30
988.	77a	AMPHI	40-43
989.	77a	AMPHI	46-52
990.	77a	AMPHI	95-97
991.	77a	AMPHI	103-107
992.	77a	AMPHI	114-125
993.	77a	AMPHI	144-151
994.	77a	AMPHI	154-156
995.	77a	AMPHI	166-184
996.	77a	Antigenic Index	7-17
997.	77a	Antigenic Index	53-67
998.	77a	Antigenic Index	74-77
999.	77a	Antigenic Index	128-131
1000.	77a	Antigenic Index	140-154
1001.	77a	Hydrophilicity	11-17
1002.	77a	Hydrophilicity	53-65
1003.	77a	Hydrophilicity	141-151

1004.	81-1	AMPHI	30-40
1005.	81-1	AMPHI	54-56
1006.	81-1	AMPHI	60-63
1007.	81-1	AMPHI	76-93
1008.	81-1	AMPHI	96-101
1009.	81-1	AMPHI	104-406
1010.	81-1	AMPHI	118-126
1011.	81-1	AMPHI	190-205
1012.	81-1	AMPHI	230-233
1013.	81-1	AMPHI	239-242
1014.	81-1	AMPHI	256-258
1015.	81-1	AMPHI	264-284
1016.	81-1	AMPHI	290-297
1017.	81-1	AMPHI	317-326
1018.	81-1	AMPHI	388-396
1019.	81-1	AMPHI	403-414
1020.	81-1	AMPHI	458-463
1021.	81-1	AMPHI	476-480
1022.	81-1	Antigenic Index	1-4
1023.	81-1	Antigenic Index	35-38
1024.	81-1	Antigenic Index	86-89
1025.	81-1	Antigenic Index	95-98
1026.	81-1	Antigenic Index	100-103
1027.	81-1	Antigenic Index	128-136
1028.	81-1	Antigenic Index	154-174
1029.	81-1	Antigenic Index	197-211
1030.	81-1	Antigenic Index	220-226
1031.	81-1	Antigenic Index	232-240
1032.	81-1	Antigenic Index	244-249
1033.	81-1	Antigenic Index	251-253
1034.	81-1	Antigenic Index	255-258
1035.	81-1	Antigenic Index	276-290
1036.	81-1	Antigenic Index	292-301
1037.	81-1	Antigenic Index	307-312
1038.	81-1	Antigenic Index	318-323
1039.	81-1	Antigenic Index	334-345
1040.	81-1	Antigenic Index	352-358
1041.	81-1	Antigenic Index	364-372
1042.	81-1	Antigenic Index	376-384
1043.	81-1	Antigenic Index	387-401

1044.	81-1	Antigenic Index	409-417
1045.	81-1	Antigenic Index	423-444
1046.	81-1	Antigenic Index	452-459
1047.	81-1	Antigenic Index	486-488
1048.	81-1	Antigenic Index	490-499
1049.	81-1	Antigenic Index	507-520
1050.	81-1	Hydrophilicity	1-4
1051.	81-1	Hydrophilicity	35-38
1052.	81-1	Hydrophilicity	95-98
1053.	81-1	Hydrophilicity	128-136
1054.	81-1	Hydrophilicity	154-164
1055.	81-1	Hydrophilicity	166-172
1056.	81-1	Hydrophilicity	202-209
1057.	81-1	Hydrophilicity	220-226
1058.	81-1	Hydrophilicity	234-238
1059.	81-1	Hydrophilicity	245-249
1060.	81-1	Hydrophilicity	251-253
1061.	81-1	Hydrophilicity	284-287
1062.	81-1	Hydrophilicity	292-299
1063.	81-1	Hydrophilicity	307-312
1064.	81-1	Hydrophilicity	321-323
1065.	81-1	Hydrophilicity	338-345
1066.	81-1	Hydrophilicity	366-368
1067.	81-1	Hydrophilicity	378-384
1068.	81-1	Hydrophilicity	387-401
1069.	81-1	Hydrophilicity	409-415
1070.	81-1	Hydrophilicity	453-459
1071.	81-1	Hydrophilicity	493-499
1072.	81-1	Hydrophilicity	507-509
1073.	81-1	Hydrophilicity	512-518
1074.	82a	AMPHI	36-40
1075.	82a	AMPHI	95-111
1076.	82a	AMPHI	117-132
1077.	82a	AMPHI	135-137
1078.	82a	AMPHI	160-174
1079.	82a	AMPHI	183-187
1080.	82a	Antigenic Index	2-8
1081.	82a	Antigenic Index	56-60
1082.	82a	Antigenic Index	90-97
1083.	82a	Antigenic Index	104-111

1084.	82a	Antigenic Index	114-137
1085.	82a	Antigenic Index	141-151
1086.	82a	Antigenic Index	170-175
1087.	82a	Antigenic Index	180-188
1088.	82a	Antigenic Index	194-201
1089.	82a	Antigenic Index	206-209
1090.	82a	Antigenic Index	216-218
1091.	82a	Hydrophilicity	2-8
1092.	82a	Hydrophilicity	56-60
1093.	82a	Hydrophilicity	90-97
1094.	82a	Hydrophilicity	105-108
1095.	82a	Hydrophilicity	120-128
1096.	82a	Hydrophilicity	130-134
1097.	82a	Hydrophilicity	141-151
1098.	82a	Hydrophilicity	170-175
1099.	82a	Hydrophilicity	186-188
1100.	82a	Hydrophilicity	195-201
1101.	82a	Hydrophilicity	206-209
1102.	112-1	AMPHI	6-8
1103.	112-1	AMPHI	12-34
1104.	112-1	AMPHI	45-53
1105.	112-1	AMPHI	63-65
1106.	112-1	AMPHI	70-82
1107.	112-1	AMPHI	84-86
1108.	112-1	AMPHI	107-109
1109.	112-1	AMPHI	116-123
1110.	112-1	AMPHI	183-186
1111.	112-1	AMPHI	244-246
~~1112.~~	~~112-1~~	~~AMPHI~~	~~248-258~~
1113.	112-1	AMPHI	280-282
1114.	112-1	AMPHI	302-313
1115.	112-1	Antigenic Index	35-44
1116.	112-1	Antigenic Index	57-61
1117.	112-1	Antigenic Index	81-84
1118.	112-1	Antigenic Index	91-98
1119.	112-1	Antigenic Index	125-133
1120.	112-1	Antigenic Index	140-147
1121.	112-1	Antigenic Index	149-159
1122.	112-1	Antigenic Index	161-165
1123.	112-1	Antigenic Index	174-190

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* 1126. *	112-1	Antigenic Index	218-224
1124.	112-1	Antigenic Index	192-200
1125.	112-1	Antigenic Index	202-216
1127.	112-1	Antigenic Index	228-232
1128.	112-1	Antigenic Index	239-244
1129.	112-1	Antigenic Index	255-263
1130.	112-1	Antigenic Index	290-300
1131.	112-1	Hydrophilicity	38-40
1132.	112-1	Hydrophilicity	57-61
1133.	112-1	Hydrophilicity	92-98
1134.	112-1	Hydrophilicity	125-133
1135.	112-1	Hydrophilicity	141-143
1136.	112-1	Hydrophilicity	150-159
1137.	112-1	Hydrophilicity	161-164
1138.	112-1	Hydrophilicity	175-190
1139.	112-1	Hydrophilicity	203-216
1140.	112-1	Hydrophilicity	218-224
1141.	112-1	Hydrophilicity	228-232
1142.	112-1	Hydrophilicity	239-244
1143.	112-1	Hydrophilicity	259-261
1144.	112-1	Hydrophilicity	293-297
1145.	112a	AMPHI	6-8
1146.	112a	AMPHI	12-34
1147.	112a	AMPHI	47-54
1148.	112a	AMPHI	63-65
1149.	112a	AMPHI	69-72
1150.	112a	AMPHI	84-86
1151.	112a	AMPHI	89-91
~~1152.~~	~~112a~~	~~AMPHI~~	~~107-109~~
1153.	112a	AMPHI	116-123
1154.	112a	AMPHI	183-186
1155.	112a	AMPHI	244-246
1156.	112a	AMPHI	248-258
1157.	112a	AMPHI	280-282
1158.	112a	AMPHI	302-310
1159.	112a	AMPHI	321-336
1160.	112a	Antigenic Index	35-44
1161.	112a	Antigenic Index	57-61
1162.	112a	Antigenic Index	81-84
1163.	112a	Antigenic Index	91-98

1164.	112a	Antigenic Index	125-133
1165.	112a	Antigenic Index	140-147
1166.	112a	Antigenic Index	150-158
1167.	112a	Antigenic Index	161-164
1168.	112a	Antigenic Index	174-190
1169.	112a	Antigenic Index	194-200
1170.	112a	Antigenic Index	202-216
1171.	112a	Antigenic Index	218-220
1172.	112a	Antigenic Index	222-224
1173.	112a	Antigenic Index	228-232
1174.	112a	Antigenic Index	239-244
1175.	112a	Antigenic Index	256-263
1176.	112a	Antigenic Index	290-301
1177.	112a	Antigenic Index	351-356
1178.	112a	Hydrophilicity	38-40
1179.	112a	Hydrophilicity	57-61
1180.	112a	Hydrophilicity	93-98
1181.	112a	Hydrophilicity	125-133
1182.	112a	Hydrophilicity	141-143
1183.	112a	Hydrophilicity	150-155
1184.	112a	Hydrophilicity	161-164
1185.	112a	Hydrophilicity	175-190
1186.	112a	Hydrophilicity	203-216
1187.	112a	Hydrophilicity	218-220
1188.	112a	Hydrophilicity	222-224
1189.	112a	Hydrophilicity	228-232
1190.	112a	Hydrophilicity	239-244
1191.	112a	Hydrophilicity	259-261
~~1192.~~	~~112a~~	~~Hydrophilicity~~	~~293-297~~
1193.	112a	Hydrophilicity	351-356
1194.	114-1	AMPHI	45-54
1195.	114-1	AMPHI	154-160
1196.	114-1	AMPHI	182-190
1197.	114-1	AMPHI	224-226
1198.	114-1	AMPHI	229-233
1199.	114-1	AMPHI	285-287
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1201.	114-1	AMPHI	321-332
1202.	114-1	AMPHI	392-398
1203.	114-1	AMPHI	413-416

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1223.	114-1	AMPHI	1111-1115
1224.	114-1	AMPHI	1163-1167
1225.	114-1	AMPHI	1242-1245
1226.	114-1	AMPHI	1275-1281
1227.	114-1	AMPHI	1312-1317
1228.	114-1	AMPHI	1338-1347
1229.	114-1	AMPHI	1349-1355
1230.	114-1	AMPHI	1357-1360
1231.	114-1	AMPHI	1362-1365
1232.	114-1	AMPHI	1376-1398
1233.	114-1	AMPHI	1418-1421
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1235.	114-1	AMPHI	1468-1473
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1237.	114-1	AMPHI	1495-1515
1238.	114-1	AMPHI	1518-1526
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1241.	114-1	AMPHI	1580-1583
1242.	114-1	AMPHI	1585-1597
1243.	114-1	AMPHI	1604-1606

1244.	114-1	AMPHI	1613-1624
1245.	114-1	AMPHI	1626-1630
1246.	114-1	AMPHI	1638-1644
1247.	114-1	AMPHI	1655-1660
1248.	114-1	AMPHI	1662-1664
1249.	114-1	AMPHI	1672-1674
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1251.	114-1	AMPHI	1691-1694
1252.	114-1	AMPHI	1713-1716
1253.	114-1	AMPHI	1719-1729
1254.	114-1	AMPHI	1735-1738
1255.	114-1	AMPHI	1753-1757
1256.	114-1	AMPHI	1772-1778
1257.	114-1	AMPHI	1790-1792
1258.	114-1	AMPHI	1817-1826
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1262.	114-1	AMPHI	1871-1881
1263.	114-1	AMPHI	1883-1896
1264.	114-1	AMPHI	1922-1927
1265.	114-1	AMPHI	1934-1946
1266.	114-1	AMPHI	1950-1955
1267.	114-1	AMPHI	1957-1964
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1276.	114-1	Antigenic Index	161-168
1277.	114-1	Antigenic Index	172-174
1278.	114-1	Antigenic Index	181-189
1279.	114-1	Antigenic Index	196-203
1280.	114-1	Antigenic Index	208-213
1281.	114-1	Antigenic Index	220-229
1282.	114-1	Antigenic Index	242-248
1283.	114-1	Antigenic Index	251-266

1284.	114-1	Antigenic Index	268-276
1285.	114-1	Antigenic Index	295-307
1286.	114-1	Antigenic Index	309-312
1287.	114-1	Antigenic Index	318-340
1288.	114-1	Antigenic Index	345-351
1289.	114-1	Antigenic Index	357-366
1290.	114-1	Antigenic Index	371-381
1291.	114-1	Antigenic Index	385-392
1292.	114-1	Antigenic Index	404-417
1293.	114-1	Antigenic Index	419-432
1294.	114-1	Antigenic Index	440-456
1295.	114-1	Antigenic Index	464-468
1296.	114-1	Antigenic Index	473-480
1297.	114-1	Antigenic Index	482-488
1298.	114-1	Antigenic Index	496-511
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1301.	114-1	Antigenic Index	555-560
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1305.	114-1	Antigenic Index	617-620
1306.	114-1	Antigenic Index	622-624
1307.	114-1	Antigenic Index	628-632
1308.	114-1	Antigenic Index	637-640
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1311.	114-1	Antigenic Index	668-688
1312.	114-1	Antigenic Index	696-725
1313.	114-1	Antigenic Index	730-733
1314.	114-1	Antigenic Index	738-755
1315.	114-1	Antigenic Index	760-766
1316.	114-1	Antigenic Index	779-783
1317.	114-1	Antigenic Index	786-799
1318.	114-1	Antigenic Index	807-809
1319.	114-1	Antigenic Index	811-819
1320.	114-1	Antigenic Index	831-839
1321.	114-1	Antigenic Index	845-857
1322.	114-1	Antigenic Index	860-862
1323.	114-1	Antigenic Index	864-868

1324.	114-1	Antigenic Index	872-879
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1326.	114-1	Antigenic Index	893-903
1327.	114-1	Antigenic Index	908-916
1328.	114-1	Antigenic Index	919-936
1329.	114-1	Antigenic Index	941-947
1330.	114-1	Antigenic Index	950-956
1331.	114-1	Antigenic Index	959-976
1332.	114-1	Antigenic Index	979-991
1333.	114-1	Antigenic Index	993-1000
1334.	114-1	Antigenic Index	1007-1022
1335.	114-1	Antigenic Index	1041-1053
1336.	114-1	Antigenic Index	1062-1068
1337.	114-1	Antigenic Index	1075-1108
1338.	114-1	Antigenic Index	1115-1121
1339.	114-1	Antigenic Index	1126-1145
1340.	114-1	Antigenic Index	1148-1152
1341.	114-1	Antigenic Index	1156-1178
1342.	114-1	Antigenic Index	1195-1206
1343.	114-1	Antigenic Index	1208-1212
1344.	114-1	Antigenic Index	1217-1243
1345.	114-1	Antigenic Index	1246-1263
1346.	114-1	Antigenic Index	1271-1282
1347.	114-1	Antigenic Index	1284-1288
1348.	114-1	Antigenic Index	1292-1295
1349.	114-1	Antigenic Index	1299-1307
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1351.	114-1	Antigenic Index	1330-1340
1352.	114-1	Antigenic Index	1344-1359
1353.	114-1	Antigenic Index	1367-1384
1354.	114-1	Antigenic Index	1395-1399
1355.	114-1	Antigenic Index	1405-1417
1356.	114-1	Antigenic Index	1445-1449
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1359.	114-1	Antigenic Index	1532-1548
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1362.	114-1	Antigenic Index	1573-1583
1363.	114-1	Antigenic Index	1594-1611

1364.	114-1	Antigenic Index	1627-1635
1365.	114-1	Antigenic Index	1643-1645
1366.	114-1	Antigenic Index	1647-1665
1367.	114-1	Antigenic Index	1680-1686
1368.	114-1	Antigenic Index	1700-1722
1369.	114-1	Antigenic Index	1724-1726
1370.	114-1	Antigenic Index	1739-1746
1371.	114-1	Antigenic Index	1752-1757
1372.	114-1	Antigenic Index	1780-1783
1373.	114-1	Antigenic Index	1791-1795
1374.	114-1	Antigenic Index	1804-1808
1375.	114-1	Antigenic Index	1829-1835
1376.	114-1	Antigenic Index	1841-1859
1377.	114-1	Antigenic Index	1867-1886
1378.	114-1	Antigenic Index	1897-1903
1379.	114-1	Antigenic Index	1908-1912
1380.	114-1	Antigenic Index	1917-1922
1381.	114-1	Antigenic Index	1926-1934
1382.	114-1	Antigenic Index	1938-1945
1383.	114-1	Antigenic Index	1947-1957
1384.	114-1	Antigenic Index	1961-1968
1385.	114-1	Antigenic Index	1974-1978
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1388.	114-1	Hydrophilicity	23-34
1389.	114-1	Hydrophilicity	43-55
1390.	114-1	Hydrophilicity	76-85
1391.	114-1	Hydrophilicity	104-110
~~1392.~~	~~114-1~~	~~Hydrophilicity~~	~~118-123~~
1393.	114-1	Hydrophilicity	127-132
1394.	114-1	Hydrophilicity	147-154
1395.	114-1	Hydrophilicity	163-167
1396.	114-1	Hydrophilicity	185-187
1397.	114-1	Hydrophilicity	197-203
1398.	114-1	Hydrophilicity	208-211
1399.	114-1	Hydrophilicity	221-227
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1402.	114-1	Hydrophilicity	263-266
1403.	114-1	Hydrophilicity	270-272

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1452.	114-1	Hydrophilicity	979-989
1453.	114-1	Hydrophilicity	993-1000
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1455.	114-1	Hydrophilicity	1041-1043
1456.	114-1	Hydrophilicity	1045-1053
1457.	114-1	Hydrophilicity	1062-1068
1458.	114-1	Hydrophilicity	1075-1078
1459.	114-1	Hydrophilicity	1080-1087
1460.	114-1	Hydrophilicity	1089-1104
1461.	114-1	Hydrophilicity	1115-1121
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1470.	114-1	Hydrophilicity	1275-1277
1471.	114-1	Hydrophilicity	1284-1288
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1483.	114-1	Hydrophilicity	1629-1635

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1489.	114-1	Hydrophilicity	1740-1746
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1492.	114-1	Hydrophilicity	1842-1855
1493.	114-1	Hydrophilicity	1876-1879
1494.	114-1	Hydrophilicity	1898-1900
1495.	114-1	Hydrophilicity	1910-1912
1496.	114-1	Hydrophilicity	1920-1922
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1503.	114a	AMPHI	182-190
1504.	114a	AMPHI	224-226
1505.	114a	AMPHI	229-233
1506.	114a	AMPHI	285-287
1507.	114a	AMPHI	303-310
1508.	114a	AMPHI	321-332
1509.	114a	AMPHI	348-350
1510.	114a	AMPHI	392-398
1511.	114a	AMPHI	414-416
1512.	114a	AMPHI	478-486
1513.	114a	AMPHI	506-509
1514.	114a	AMPHI	525-529
1515.	114a	AMPHI	565-567
1516.	114a	AMPHI	614-621
1517.	114a	AMPHI	631-635
1518.	114a	AMPHI	770-774
1519.	114a	AMPHI	811-813
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1522.	114a	AMPHI	875-879
1523.	114a	AMPHI	951-959

1524.	114a	AMPHI	975-981
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1526.	114a	AMPHI	1048-1051
1527.	114a	AMPHI	1073-1081
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1532.	114a	AMPHI	1275-1281
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1536.	114a	AMPHI	1357-1365
1537.	114a	AMPHI	1376-1398
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1542.	114a	AMPHI	1507-1512
1543.	114a	Antigenic Index	1-6
1544.	114a	Antigenic Index	10-16
1545.	114a	Antigenic Index	23-37
1546.	114a	Antigenic Index	41-55
1547.	114a	Antigenic Index	75-85
1548.	114a	Antigenic Index	91-97
1549.	114a	Antigenic Index	102-137
1550.	114a	Antigenic Index	147-156
1551.	114a	Antigenic Index	161-168
1552.	114a	Antigenic Index	172-174
1553.	114a	Antigenic Index	181-189
1554.	114a	Antigenic Index	196-203
1555.	114a	Antigenic Index	208-213
1556.	114a	Antigenic Index	220-229
1557.	114a	Antigenic Index	242-248
1558.	114a	Antigenic Index	251-266
1559.	114a	Antigenic Index	268-276
1560.	114a	Antigenic Index	295-307
1561.	114a	Antigenic Index	309-312
1562.	114a	Antigenic Index	318-340
1563.	114a	Antigenic Index	345-352

1564.	114a	Antigenic Index	357-366
1565.	114a	Antigenic Index	371-381
1566.	114a	Antigenic Index	385-392
1567.	114a	Antigenic Index	404-427
1568.	114a	Antigenic Index	429-434
1569.	114a	Antigenic Index	440-456
1570.	114a	Antigenic Index	465-468
1571.	114a	Antigenic Index	473-494
1572.	114a	Antigenic Index	496-510
1573.	114a	Antigenic Index	515-530
1574.	114a	Antigenic Index	535-549
1575.	114a	Antigenic Index	555-560
1576.	114a	Antigenic Index	564-578
1577.	114a	Antigenic Index	588-596
1578.	114a	Antigenic Index	602-615
1579.	114a	Antigenic Index	617-620
1580.	114a	Antigenic Index	622-624
1581.	114a	Antigenic Index	628-632
1582.	114a	Antigenic Index	637-640
1583.	114a	Antigenic Index	647-654
1584.	114a	Antigenic Index	660-666
1585.	114a	Antigenic Index	668-688
1586.	114a	Antigenic Index	697-725
1587.	114a	Antigenic Index	730-733
1588.	114a	Antigenic Index	738-755
1589.	114a	Antigenic Index	760-766
1590.	114a	Antigenic Index	779-783
1591.	114a	Antigenic Index	786-799
1592.	114a	Antigenic Index	806-809
1593.	114a	Antigenic Index	811-819
1594.	114a	Antigenic Index	831-839
1595.	114a	Antigenic Index	845-857
1596.	114a	Antigenic Index	860-862
1597.	114a	Antigenic Index	864-868
1598.	114a	Antigenic Index	872-879
1599.	114a	Antigenic Index	883-891
1600.	114a	Antigenic Index	893-902
1601.	114a	Antigenic Index	908-916
1602.	114a	Antigenic Index	923-936
1603.	114a	Antigenic Index	941-947

1604.	114a	Antigenic Index	950-956
1605.	114a	Antigenic Index	959-976
1606.	114a	Antigenic Index	979-989
1607.	114a	Antigenic Index	993-1000
1608.	114a	Antigenic Index	1007-1022
1609.	114a	Antigenic Index	1041-1053
1610.	114a	Antigenic Index	1062-1068
1611.	114a	Antigenic Index	1075-1108
1612.	114a	Antigenic Index	1115-1121
1613.	114a	Antigenic Index	1126-1145
1614.	114a	Antigenic Index	1148-1152
1615.	114a	Antigenic Index	1157-1176
1616.	114a	Antigenic Index	1195-1206
1617.	114a	Antigenic Index	1208-1212
1618.	114a	Antigenic Index	1224-1243
1619.	114a	Antigenic Index	1247-1263
1620.	114a	Antigenic Index	1271-1282
1621.	114a	Antigenic Index	1284-1288
1622.	114a	Antigenic Index	1292-1295
1623.	114a	Antigenic Index	1299-1307
1624.	114a	Antigenic Index	1318-1328
1625.	114a	Antigenic Index	1330-1340
1626.	114a	Antigenic Index	1344-1359
1627.	114a	Antigenic Index	1367-1384
1628.	114a	Antigenic Index	1396-1399
1629.	114a	Antigenic Index	1405-1417
1630.	114a	Antigenic Index	1434-1436
1631.	114a	Antigenic Index	1449-1451
~~1632.~~	~~114a~~	~~Antigenic Index~~	~~1468-1487~~
1633.	114a	Antigenic Index	1498-1503
1634.	114a	Antigenic Index	1509-1515
1635.	114a	Antigenic Index	1525-1532
1636.	114a	Hydrophilicity	4-6
1637.	114a	Hydrophilicity	12-15
1638.	114a	Hydrophilicity	23-34
1639.	114a	Hydrophilicity	43-55
1640.	114a	Hydrophilicity	75-85
1641.	114a	Hydrophilicity	104-110
1642.	114a	Hydrophilicity	118-123
1643.	114a	Hydrophilicity	127-132

1644.	114a	Hydrophilicity	147-154
1645.	114a	Hydrophilicity	163-167
1646.	114a	Hydrophilicity	185-187
1647.	114a	Hydrophilicity	197-203
1648.	114a	Hydrophilicity	208-211
1649.	114a	Hydrophilicity	221-227
1650.	114a	Hydrophilicity	243-245
1651.	114a	Hydrophilicity	253-261
1652.	114a	Hydrophilicity	263-266
1653.	114a	Hydrophilicity	270-272
1654.	114a	Hydrophilicity	295-301
1655.	114a	Hydrophilicity	309-312
1656.	114a	Hydrophilicity	320-328
1657.	114a	Hydrophilicity	332-337
1658.	114a	Hydrophilicity	345-351
1659.	114a	Hydrophilicity	360-366
1660.	114a	Hydrophilicity	371-378
1661.	114a	Hydrophilicity	387-392
1662.	114a	Hydrophilicity	404-417
1663.	114a	Hydrophilicity	421-423
1664.	114a	Hydrophilicity	425-427
1665.	114a	Hydrophilicity	442-456
1666.	114a	Hydrophilicity	473-488
1667.	114a	Hydrophilicity	499-509
1668.	114a	Hydrophilicity	515-520
1669.	114a	Hydrophilicity	536-549
1670.	114a	Hydrophilicity	555-560
1671.	114a	Hydrophilicity	565-568
~~1672.~~	~~114a~~	~~Hydrophilicity~~	~~570-578~~
1673.	114a	Hydrophilicity	589-594
1674.	114a	Hydrophilicity	602-604
1675.	114a	Hydrophilicity	609-615
1676.	114a	Hydrophilicity	617-620
1677.	114a	Hydrophilicity	660-665
1678.	114a	Hydrophilicity	668-680
1679.	114a	Hydrophilicity	684-686
1680.	114a	Hydrophilicity	699-708
1681.	114a	Hydrophilicity	715-725
1682.	114a	Hydrophilicity	730-733
1683.	114a	Hydrophilicity	738-744

1684.	114a	Hydrophilicity	746-754
1685.	114a	Hydrophilicity	760-766
1686.	114a	Hydrophilicity	789-793
1687.	114a	Hydrophilicity	816-818
1688.	114a	Hydrophilicity	831-836
1689.	114a	Hydrophilicity	845-857
1690.	114a	Hydrophilicity	860-862
1691.	114a	Hydrophilicity	864-866
1692.	114a	Hydrophilicity	873-879
1693.	114a	Hydrophilicity	883-885
1694.	114a	Hydrophilicity	887-889
1695.	114a	Hydrophilicity	896-899
1696.	114a	Hydrophilicity	908-916
1697.	114a	Hydrophilicity	923-932
1698.	114a	Hydrophilicity	941-947
1699.	114a	Hydrophilicity	961-975
1700.	114a	Hydrophilicity	979-989
1701.	114a	Hydrophilicity	993-1000
1702.	114a	Hydrophilicity	1007-1022
1703.	114a	Hydrophilicity	1041-1043
1704.	114a	Hydrophilicity	1045-1053
1705.	114a	Hydrophilicity	1062-1068
1706.	114a	Hydrophilicity	1075-1078
1707.	114a	Hydrophilicity	1080-1087
1708.	114a	Hydrophilicity	1089-1104
1709.	114a	Hydrophilicity	1115-1121
1710.	114a	Hydrophilicity	1126-1141
1711.	114a	Hydrophilicity	1143-1145
~~1712.~~	~~114a~~	~~Hydrophilicity~~	~~1148-1151~~
1713.	114a	Hydrophilicity	1158-1171
1714.	114a	Hydrophilicity	1197-1203
1715.	114a	Hydrophilicity	1224-1243
1716.	114a	Hydrophilicity	1251-1263
1717.	114a	Hydrophilicity	1271-1273
1718.	114a	Hydrophilicity	1275-1277
1719.	114a	Hydrophilicity	1284-1288
1720.	114a	Hydrophilicity	1299-1307
1721.	114a	Hydrophilicity	1318-1326
1722.	114a	Hydrophilicity	1334-1340
1723.	114a	Hydrophilicity	1350-1359

1724.	114a	Hydrophilicity	1367-1384
1725.	114a	Hydrophilicity	1407-1417
1726.	114a	Hydrophilicity	1449-1451
1727.	114a	Hydrophilicity	1469-1482
1728.	114a	Hydrophilicity	1484-1486
1729.	114a	Hydrophilicity	1498-1503
1730.	114a	Hydrophilicity	1510-1512
1731.	114a	Hydrophilicity	1527-1532
1732.	124-1	AMPHI	37-43
1733.	124-1	AMPHI	94-96
1734.	124-1	AMPHI	113-115
1735.	124-1	Antigenic Index	20-26
1736.	124-1	Antigenic Index	38-43
1737.	124-1	Antigenic Index	52-55
1738.	124-1	Antigenic Index	62-70
1739.	124-1	Antigenic Index	88-97
1740.	124-1	Antigenic Index	104-114
1741.	124-1	Antigenic Index	123-135
1742.	124-1	Antigenic Index	146-155
1743.	124-1	Hydrophilicity	20-26
1744.	124-1	Hydrophilicity	41-43
1745.	124-1	Hydrophilicity	52-55
1746.	124-1	Hydrophilicity	63-69
1747.	124-1	Hydrophilicity	91-94
1748.	124-1	Hydrophilicity	104-114
1749.	124-1	Hydrophilicity	123-135
1750.	124-1	Hydrophilicity	146-155
1751.	124a	AMPHI	19-21
1752.	124a	AMPHI	23-29
1753.	124a	AMPHI	37-43
1754.	124a	AMPHI	94-96
1755.	124a	Antigenic Index	38-43
1756.	124a	Antigenic Index	52-55
1757.	124a	Antigenic Index	62-70
1758.	124a	Antigenic Index	77-80
1759.	124a	Antigenic Index	90-96
1760.	124a	Antigenic Index	105-115
1761.	124a	Antigenic Index	120-135
1762.	124a	Antigenic Index	145-153
1763.	124a	Hydrophilicity	41-43

1764.	124a	Hydrophilicity	52-55
1765.	124a	Hydrophilicity	63-69
1766.	124a	Hydrophilicity	91-95
1767.	124a	Hydrophilicity	108-115
1768.	124a	Hydrophilicity	120-135
1769.	124a	Hydrophilicity	146-153

It will be understood that the invention is described above by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

TABLE II

The present invention does not include within its scope proteins comprising any of the 45 protein sequences disclosed in Annex I. As stated above, if the length of any particular protein sequence disclosed in PCT/IB99/00103 is *x* amino acids, the antigenic fragment of the present invention has at most *x-1* amino acids of that protein. For each of the 45 protein sequences given in Annex 1, the value of *x* is given in the following table:

SEQ ID NO:	*x*	SEQ ID NO:	*x*	SEQ ID NO:	*x*	SEQ ID NO:	*X*
2	245	26	571	50	185	74	150
4	591	28	710	52	166	76	255
6	592	30	710	54	326	78	255
8	164	32	62	56	356	80	172
10	321	34	86	58	284	82	242
12	321	36	92	60	1978	84	242
14	124	38	103	62	1532	86	183
16	124	40	85	64	593	88	155
18	173	42	78	66	129	90	153
20	640	44	78	68	319		
22	761	46	219	70	619		
24	111	48	212	72	595		

ANNEX I

COPY OF

INTERNATIONAL PATENT APPLICATION

PCT/IB99/00103

MENINGOCOCCAL ANTIGENS

This invention relates to antigens from the bacterium *Neisseria meningitidis*.

BACKGROUND

Neisseria meningitidis is a non-motile, gram negative diplococcus human pathogen. It colonises the pharynx, causing meningitis and, occasionally, septicaemia in the absence of meningitis. It is closely related to *N.gonorrhoeae*, although one feature that clearly differentiates meningococcus from gonococcus is the presence of a polysaccharide capsule that is present in all pathogenic meningococci.

N.meningitidis causes both endemic and epidemic disease. In the United States the attack rate is 0.6-1 per 100,000 persons per year, and it can be much greater during outbreaks (see Lieberman *et al.* (1996) Safety and Immunogenicity of a Serogroups A/C *Neisseria meningitidis* Oligosaccharide-Protein Conjugate Vaccine in Young Children. *JAMA* 275(19):1499-1503; Schuchat *et al* (1997) Bacterial Meningitis in the United States in 1995. *N Engl J Med* 337(14):970-976). In developing countries, endemic disease rates are much higher and during epidemics incidence rates can reach 500 cases per 100,000 persons per year. Mortality is extremely high, at 10-20% in the United States, and much higher in developing countries. Following the introduction of the conjugate vaccine against *Haemophilus influenzae*, *N. meningitidis* is the major cause of bacterial meningitis at all ages in the United States (Schuchat *et al* (1997) *supra*).

Based on the organism's capsular polysaccharide, 12 serogroups of *N.meningitidis* have been identified. Group A is the pathogen most often implicated in epidemic disease in sub-Saharan Africa. Serogroups B and C are responsible for the vast majority of cases in the United States and in most developed countries. Serogroups W135 and Y are responsible for the rest of the cases in the United States and developed countries. The meningococcal vaccine currently in use is a tetravalent polysaccharide vaccine composed of serogroups A, C, Y and W135. Although efficacious in adolescents and adults, it induces a poor immune response and short duration of protection, and cannot be used in infants [*eg.* Morbidity and Mortality weekly report, Vol.46, No. RR-5 (1997)]. This is because polysaccharides are T-cell independent antigens that induce a weak

immune response that cannot be boosted by repeated immunization. Following the success of the vaccination against *H.influenzae*, conjugate vaccines against serogroups A and C have been developed and are at the final stage of clinical testing (Zollinger WD "New and Improved Vaccines Against Meningococcal Disease" in: *New Generation Vaccines*, *supra*, pp. 469-488; Lieberman *et al* (1996) *supra*; Costantino *et al* (1992) Development and phase I clinical testing of a conjugate vaccine against meningococcus A and C. *Vaccine* 10:691-698).

Meningococcus B remains a problem, however. This serotype currently is responsible for approximately 50% of total meningitis in the United States, Europe, and South America. The polysaccharide approach cannot be used because the menB capsular polysaccharide is a polymer of α(2-8)-linked *N*-acetyl neuraminic acid that is also present in mammalian tissue. This results in tolerance to the antigen; indeed, if an immune response were elicited, it would be anti-self, and therefore undesirable. In order to avoid induction of autoimmunity and to induce a protective immune response, the capsular polysaccharide has, for instance, been chemically modified substituting the *N*-acetyl groups with *N*-propionyl groups, leaving the specific antigenicity unaltered (Romero & Outschoorn (1994) Current status of Meningococcal group B vaccine candidates: capsular or non-capsular? *Clin Microbiol Rev* 7(4):559-575).

Alternative approaches to menB vaccines have used complex mixtures of outer membrane proteins (OMPs), containing either the OMPs alone, or OMPs enriched in porins, or deleted of the class 4 OMPs that are believed to induce antibodies that block bactericidal activity. This approach produces vaccines that are not well characterized. They are able to protect against the homologous strain, but are not effective at large where there are many antigenic variants of the outer membrane proteins. To overcome the antigenic variability, multivalent vaccines containing up to nine different porins have been constructed (*eg.* Poolman JT (1992) Development of a meningococcal vaccine. *Infect. Agents Dis.* 4:13-28). Additional proteins to be used in outer membrane vaccines have been the opa and opc proteins, but none of these approaches have been able to overcome the antigenic variability (*eg.* Ala'Aldeen & Borriello (1996) The meningococcal transferrin-binding proteins 1 and 2 are both surface exposed and generate bactericidal antibodies capable of killing homologous and heterologous strains. *Vaccine* 14(1):49-53).

A certain amount of sequence data is available for meningococcal and gonococcal genes and proteins (*eg.* EP-A-0467714, WO96/29412), but this is by no means complete. The provision of

further sequences could provide an opportunity to identify secreted or surface-exposed proteins that are presumed targets for the immune system and which are not antigenically variable. For instance, some of the identified proteins could be components of efficacious vaccines against meningococcus B, some could be components of vaccines against all meningococcal serotypes, and others could be components of vaccines against all pathogenic *Neisseriae*.

THE INVENTION

The invention provides proteins comprising the *N.meningitidis* amino acid sequences disclosed in the examples.

It also provides proteins comprising sequences homologous (*ie.* having sequence identity) to the *N.meningitidis* amino acid sequences disclosed in the examples. Depending on the particular sequence, the degree of sequence identity is preferably greater than 50% (*eg.* 60%, 70%, 80%, 90%, 95%, 99% or more). These homologous proteins include mutants and allelic variants of the sequences disclosed in the examples. Typically, 50% identity or more between two proteins is considered to be an indication of functional equivalence. Identity between proteins is preferably determined by the Smith-Waterman homology search algorithm as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with parameters *gap open penalty=12* and *gap extension penalty=1*.

The invention further provides proteins comprising fragments of the *N.meningitidis* amino acid sequences disclosed in the examples. The fragments should comprise at least *n* consecutive amino acids from the sequences and, depending on the particular sequence, *n* is 7 or more (*eg.* 8, 10, 12, 14, 16, 18, 20 or more). Preferably the fragments comprise an epitope from the sequence.

The proteins of the invention can, of course, be prepared by various means (*eg.* recombinant expression, purification from cell culture, chemical synthesis *etc.*) and in various forms (*eg.* native, fusions *etc.*). They are preferably prepared in substantially pure form (*ie.* substantially free from other *N.meningitidis* or host cell proteins)

According to a further aspect, the invention provides antibodies which bind to these proteins. These may be polyclonal or monoclonal and may be produced by any suitable means.

According to a further aspect, the invention provides nucleic acid comprising the *N.meningitidis* nucleotide sequences disclosed in the examples. In addition, the invention provides nucleic acid comprising sequences homologous (*ie.* having sequence identity) to the *N.meningitidis* nucleotide sequences disclosed in the examples.

Furthermore, the invention provides nucleic acid which can hybridise to the *N.meningitidis* nucleic acid disclosed in the examples, preferably under "high stringency" conditions (*eg.* 65°C in a 0.1xSSC, 0.5% SDS solution).

Nucleic acid comprising fragments of these sequences are also provided. These should comprise at least *n* consecutive nucleotides from the *N.meningitidis* sequences and, depending on the particular sequence, *n* is 10 or more (*eg* 12, 14, 15, 18, 20, 25, 30, 35, 40 or more).

According to a further aspect, the invention provides nucleic acid encoding the proteins and protein fragments of the invention.

It should also be appreciated that the invention provides nucleic acid comprising sequences complementary to those described above (*eg.* for antisense or probing purposes).

Nucleic acid according to the invention can, of course, be prepared in many ways (*eg.* by chemical synthesis, from genomic or cDNA libraries, from the organism itself *etc.*) and can take various forms (*eg.* single stranded, double stranded, vectors, probes *etc.*).

In addition, the term "nucleic acid" includes DNA and RNA, and also their analogues, such as those containing modified backbones, and also peptide nucleic acids (PNA) *etc.*

According to a further aspect, the invention provides vectors comprising nucleotide sequences of the invention (*eg.* expression vectors) and host cells transformed with such vectors.

According to a further aspect, the invention provides compositions comprising protein, antibody, and/or nucleic acid according to the invention. These compositions may be suitable as vaccines, for instance, or as diagnostic reagents, or as immunogenic compositions.

The invention also provides nucleic acid, protein, or antibody according to the invention for use as medicaments (*eg.* as vaccines) or as diagnostic reagents. It also provides the use of nucleic acid, protein, or antibody according to the invention in the manufacture of: (i) a medicament for treating or preventing infection due to Neisserial bacteria; (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria; and/or (iii) a reagent which can raise antibodies against Neisserial bacteria. Said Neisserial bacteria may be any species or strain (such as *N.gonorrhoeae*) but are preferably *N.meningitidis*, especially strain A, strain B or strain C.

The invention also provides a method of treating a patient, comprising administering to the patient a therapeutically effective amount of nucleic acid, protein, and/or antibody according to the invention.

According to further aspects, the invention provides various processes.

A process for producing proteins of the invention is provided, comprising the step of culturing a host cell according to the invention under conditions which induce protein expression.

A process for producing protein or nucleic acid of the invention is provided, wherein the protein or nucleic acid is synthesised in part or in whole using chemical means.

A process for detecting polynucleotides of the invention is provided, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b) detecting said duplexes.

A process for detecting proteins of the invention is provided, comprising the steps of: (a) contacting an antibody according to the invention with a biological sample under conditions suitable for the formation of an antibody-antigen complexes; and (b) detecting said complexes.

Unlike the sequences disclosed in PCT/IB98/01665, the sequences disclosed in the present application are believed not to have any significant homologs in *N.gonorrhoeae*. Accordingly, the sequences of the present invention also find use in the preparation of reagents for distinguishing between *N.meningitidis* and *N.gonorrhoeae*

A summary of standard techniques and procedures which may be employed in order to perform the invention (*eg.* to utilise the disclosed sequences for vaccination or diagnostic purposes) follows. This summary is not a limitation on the invention but, rather, gives examples that may be used, but are not required.

General

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature eg. Sambrook *Molecular Cloning; A Laboratory Manual, Second Edition* (1989); *DNA Cloning, Volumes I and II* (D.N Glover ed. 1985); *Oligonucleotide Synthesis* (M.J. Gait ed, 1984); *Nucleic Acid Hybridization* (B.D. Hames & S.J. Higgins eds. 1984); *Transcription and Translation* (B.D. Hames & S.J. Higgins eds. 1984); *Animal Cell Culture* (R.I. Freshney ed. 1986); *Immobilized Cells and Enzymes* (IRL Press, 1986); B. Perbal, *A Practical Guide to Molecular Cloning* (1984); the *Methods in Enzymology* series (Academic Press. Inc.), especially volumes 154 & 155; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Mayer and Walker, eds. (1987), *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); Scopes, (1987) *Protein Purification: Principles and Practice*, Second Edition (Springer-Verlag, N.Y.), and *Handbook of Experimental Immunology, Volumes I-IV* (D.M. Weir and C. C. Blackwell eds 1986).

Standard abbreviations for nucleotides and amino acids are used in this specification.

All publications, patents, and patent applications cited herein are incorporated in full by reference In particular, the contents of UK patent applications 9800760.2, 9819015.0 and 9822143.5 are incorporated herein.

Definitions

A composition containing X is "substantially free of" Y when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about 95% or even 99% by weight.

The term "comprising" means "including" as well as "consisting" *eg.* a composition "comprising" X may consist exclusively of X or may include something additional to X, such as X+Y.

The term "heterologous" refers to two biological components that are not found together in nature. The components may be host cells, genes, or regulatory regions, such as promoters. Although the heterologous components are not found together in nature, they can function together, as when a promoter heterologous to a gene is operably linked to the gene. Another example is where a Neisserial sequence is heterologous to a mouse host cell. A further examples would be two epitopes from the same or different proteins which have been assembled in a single protein in an arrangement not found in nature.

An "origin of replication" is a polynucleotide sequence that initiates and regulates replication of polynucleotides, such as an expression vector. The origin of replication behaves as an autonomous unit of polynucleotide replication within a cell, capable of replication under its own control. An origin of replication may be needed for a vector to replicate in a particular host cell. With certain origins of replication, an expression vector can be reproduced at a high copy number in the presence of the appropriate proteins within the cell. Examples of origins are the autonomously replicating sequences, which are effective in yeast; and the viral T-antigen, effective in COS-7 cells.

A "mutant" sequence is defined as DNA, RNA or amino acid sequence differing from but having sequence identity with the native or disclosed sequence. Depending on the particular sequence, the degree of sequence identity between the native or disclosed sequence and the mutant sequence is preferably greater than 50% (*eg.* 60%, 70%, 80%, 90%, 95%, 99% or more, calculated using the Smith-Waterman algorithm as described above). As used herein, an "allelic variant" of a nucleic acid molecule, or region, for which nucleic acid sequence is provided herein is a nucleic acid molecule, or region, that occurs essentially at the same locus in the genome of another or second isolate, and that, due to natural variation caused by, for example, mutation or recombination, has a similar but not identical nucleic acid sequence. A coding region allelic variant typically encodes a protein having similar activity to that of the protein encoded by the gene to which it is being compared. An allelic variant can also comprise an alteration in the 5' or 3' untranslated regions of the gene, such as in regulatory control regions (*eg.* see US. patent 5,753,235).

Expression systems

The Neisserial nucleotide sequences can be expressed in a variety of different expression systems; for example those used with mammalian cells, baculoviruses, plants, bacteria, and yeast.

i. Mammalian Systems

Mammalian expression systems are known in the art. A mammalian promoter is any DNA sequence capable of binding mammalian RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiating region, which is usually placed proximal to the 5' end of the coding sequence, and a TATA box, usually located 25-30 base pairs (bp) upstream of the transcription initiation site. The TATA box is thought to direct RNA polymerase II to begin RNA synthesis at the correct site. A mammalian promoter will also contain an upstream promoter element, usually located within 100 to 200 bp upstream of the TATA box. An upstream promoter element determines the rate at which transcription is initiated and can act in either orientation [Sambrook et al. (1989) "Expression of Cloned Genes in Mammalian Cells." In *Molecular Cloning: A Laboratory Manual, 2nd ed.*].

Mammalian viral genes are often highly expressed and have a broad host range; therefore sequences encoding mammalian viral genes provide particularly useful promoter sequences. Examples include the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter (Ad MLP), and herpes simplex virus promoter. In addition, sequences derived from non-viral genes, such as the murine metallotheionein gene, also provide useful promoter sequences. Expression may be either constitutive or regulated (inducible), depending on the promoter can be induced with glucocorticoid in hormone-responsive cells.

The presence of an enhancer element (enhancer), combined with the promoter elements described above, will usually increase expression levels. An enhancer is a regulatory DNA sequence that can stimulate transcription up to 1000-fold when linked to homologous or heterologous promoters, with synthesis beginning at the normal RNA start site. Enhancers are also active when they are placed upstream or downstream from the transcription initiation site, in either normal or flipped orientation, or at a distance of more than 1000 nucleotides from the promoter [Maniatis et al. (1987) *Science 236*:1237; Alberts et al. (1989) *Molecular Biology of the Cell*, 2nd ed.]. Enhancer elements derived from viruses may be particularly useful, because they usually have a broader host

range. Examples include the SV40 early gene enhancer [Dijkema et al (1985) *EMBO J. 4*:761] and the enhancer/promoters derived from the long terminal repeat (LTR) of the Rous Sarcoma Virus [Gorman et al. (1982b) *Proc. Natl. Acad. Sci. 79*:6777] and from human cytomegalovirus [Boshart et al. (1985) *Cell 41*:521]. Additionally, some enhancers are regulatable and become active only in the presence of an inducer, such as a hormone or metal ion [Sassone-Corsi and Borelli (1986) *Trends Genet. 2*:215; Maniatis et al. (1987) *Science* 236:1237].

A DNA molecule may be expressed intracellularly in mammalian cells. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in mammalian cells. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in vitro*. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The adenovirus triparite leader is an example of a leader sequence that provides for secretion of a foreign protein in mammalian cells.

Usually, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. The 3' terminus of the mature mRNA is formed by site-specific post-transcriptional cleavage and polyadenylation [Birnstiel et al. (1985) *Cell 41*:349; Proudfoot and Whitelaw (1988) "Termination and 3' end processing of eukaryotic RNA. In *Transcription and splicing* (ed. B.D. Hames and D.M. Glover); Proudfoot (1989) *Trends Biochem. Sci. 14*:105]. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminater/polyadenylation signals include those derived from SV40 [Sambrook et al (1989) "Expression of cloned genes in cultured mammalian cells." In *Molecular Cloning: A Laboratory Manual*].

Usually, the above described components, comprising a promoter, polyadenylation signal, and transcription termination sequence are put together into expression constructs. Enhancers, introns with functional splice donor and acceptor sites, and leader sequences may also be included in an expression construct, if desired. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as mammalian cells or bacteria. Mammalian replication systems include those derived from animal viruses, which require trans-acting factors to replicate. For example, plasmids containing the replication systems of papovaviruses, such as SV40 [Gluzman (1981) *Cell 23*:175] or polyomavirus, replicate to extremely high copy number in the presence of the appropriate viral T antigen. Additional examples of mammalian replicons include those derived from bovine papillomavirus and Epstein-Barr virus. Additionally, the replicon may have two replicaton systems, thus allowing it to be maintained, for example, in mammalian cells for expression and in a prokaryotic host for cloning and amplification. Examples of such mammalian-bacteria shuttle vectors include pMT2 [Kaufman et al. (1989) *Mol. Cell. Biol. 9*:946] and pHEBO [Shimizu et al. (1986) *Mol. Cell. Biol. 6*:1074].

The transformation procedure used depends upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), including but not limited to, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*eg.* Hep G2), and a number of other cell lines.

ii. Baculovirus Systems

The polynucleotide encoding the protein can also be inserted into a suitable insect expression vector, and is operably linked to the control elements within that vector. Vector construction employs techniques which are known in the art. Generally, the components of the expression system include a transfer vector, usually a bacterial plasmid, which contains both a fragment of the baculovirus genome, and a

convenient restriction site for insertion of the heterologous gene or genes to be expressed; a wild type baculovirus with a sequence homologous to the baculovirus-specific fragment in the transfer vector (this allows for the homologous recombination of the heterologous gene in to the baculovirus genome); and appropriate insect host cells and growth media.

After inserting the DNA sequence encoding the protein into the transfer vector, the vector and the wild type viral genome are transfected into an insect host cell where the vector and viral genome are allowed to recombine. The packaged recombinant virus is expressed and recombinant plaques are identified and purified. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *inter alia*, Invitrogen, San Diego CA ("MaxBac" kit). These techniques are generally known to those skilled in the art and fully described in Summers and Smith, *Texas Agricultural Experiment Station Bulletin No. 1555* (1987) (hereinafter "Summers and Smith").

Prior to inserting the DNA sequence encoding the protein into the baculovirus genome, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are usually assembled into an intermediate transplacement construct (transfer vector). This construct may contain a single gene and operably linked regulatory elements; multiple genes, each with its owned set of operably linked regulatory elements; or multiple genes, regulated by the same set of regulatory elements. Intermediate transplacement constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as a bacterium. The replicon will have a replication system, thus allowing it to be maintained in a suitable host for cloning and amplification.

Currently, the most commonly used transfer vector for introducing foreign genes into AcNPV is pAc373. Many other vectors, known to those of skill in the art, have also been designed. These include, for example, pVL985 (which alters the polyhedrin start codon from ATG to ATT, and which introduces a BamHI cloning site 32 basepairs downstream from the ATT; see Luckow and Summers, *Virology* (1989) *17*:31.

The plasmid usually also contains the polyhedrin polyadenylation signal (Miller et al. (1988) *Ann. Rev. Microbiol.*, *42*:177) and a prokaryotic ampicillin-resistance (*amp*) gene and origin of replication for selection and propagation in *E. coli*.

Baculovirus transfer vectors usually contain a baculovirus promoter. A baculovirus promoter is any DNA sequence capable of binding a baculovirus RNA polymerase and initiating the downstream (5' to 3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A baculovirus transfer vector may also have a second domain called an enhancer, which, if present, is usually distal to the structural gene. Expression may be either regulated or constitutive.

Structural genes, abundantly transcribed at late times in a viral infection cycle, provide particularly useful promoter sequences. Examples include sequences derived from the gene encoding the viral polyhedron protein, Friesen et al., (1986) "The Regulation of Baculovirus Gene Expression," in: *The Molecular Biology of Baculoviruses* (ed. Walter Doerfler); EPO Publ. Nos. 127 839 and 155 476; and the gene encoding the p10 protein, Vlak et al., (1988), *J. Gen. Virol. 69*:765.

DNA encoding suitable signal sequences can be derived from genes for secreted insect or baculovirus proteins, such as the baculovirus polyhedrin gene (Carbonell et al. (1988) *Gene*, *73*:409). Alternatively, since the signals for mammalian cell posttranslational modifications (such as signal peptide cleavage, proteolytic cleavage, and phosphorylation) appear to be recognized by insect cells, and the signals required for secretion and nuclear accumulation also appear to be conserved between the invertebrate cells and vertebrate cells, leaders of non-insect origin, such as those derived from genes encoding human α-interferon, Maeda et al., (1985), *Nature 315*:592; human gastrin-releasing peptide, Lebacq-Verheyden et al., (1988), *Molec. Cell. Biol. 8*:3129; human IL-2, Smith et al., (1985) *Proc. Nat'l Acad. Sci. USA*, *82*:8404; mouse IL-3, (Miyajima et al., (1987) *Gene 58*:273; and human glucocerebrosidase, Martin et al. (1988) *DNA*, *7*:99, can also be used to provide for secretion in insects.

A recombinant polypeptide or polyprotein may be expressed intracellularly or, if it is expressed with the proper regulatory sequences, it can be secreted. Good intracellular expression of nonfused foreign proteins usually requires heterologous genes that ideally have a short leader sequence containing suitable translation initiation signals preceding an ATG start signal. If desired, methionine at the N-terminus may be cleaved from the mature protein by *in vitro* incubation with cyanogen bromide.

Alternatively, recombinant polyproteins or proteins which are not naturally secreted can be secreted from the insect cell by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provides for secretion of the foreign protein in insects. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the translocation of the protein into the endoplasmic reticulum.

After insertion of the DNA sequence and/or the gene encoding the expression product precursor of the protein, an insect cell host is co-transformed with the heterologous DNA of the transfer vector and the genomic DNA of wild type baculovirus -- usually by co-transfection. The promoter and transcription termination sequence of the construct will usually comprise a 2-5kb section of the baculovirus genome. Methods for introducing heterologous DNA into the desired site in the baculovirus virus are known in the art. (See Summers and Smith *supra*; Ju et al. (1987); Smith et al., *Mol. Cell. Biol.* (1983) *3*:2156; and Luckow and Summers (1989)). For example, the insertion can be into a gene such as the polyhedrin gene, by homologous double crossover recombination; insertion can also be into a restriction enzyme site engineered into the desired baculovirus gene. Miller et al., (1989), *Bioessays 4*:91. The DNA sequence, when cloned in place of the polyhedrin gene in the expression vector, is flanked both 5' and 3' by polyhedrin-specific sequences and is positioned downstream of the polyhedrin promoter.

The newly formed baculovirus expression vector is subsequently packaged into an infectious recombinant baculovirus. Homologous recombination occurs at low frequency (between about 1% and about 5%); thus, the majority of the virus produced after cotransfection is still wild-type virus. Therefore, a method is necessary to identify recombinant viruses. An advantage of the expression system is a visual screen allowing recombinant viruses to be distinguished. The polyhedrin protein, which is produced by the native virus, is produced at very high levels in the nuclei of infected cells at late times after viral infection. Accumulated polyhedrin protein forms occlusion bodies that also contain embedded particles. These occlusion bodies, up to 15 μm in size, are highly refractile, giving them a bright shiny appearance that is readily visualized under the light microscope. Cells infected with recombinant viruses lack occlusion bodies. To distinguish recombinant virus from wild-type virus, the transfection supernatant is plaqued onto a monolayer of insect cells by techniques known to those skilled in the art. Namely, the plaques are screened under the light microscope for the presence (indicative of wild-type virus) or absence (indicative

of recombinant virus) of occlusion bodies. "Current Protocols in Microbiology" Vol. 2 (Ausubel et al. eds) at 16.8 (Supp. 10, 1990); Summers and Smith, *supra*; Miller et al. (1989).

Recombinant baculovirus expression vectors have been developed for infection into several insect cells. For example, recombinant baculoviruses have been developed for, *inter alia*: *Aedes aegypti*, *Autographa californica*, *Bombyx mori*, *Drosophila melanogaster*, *Spodoptera frugiperda*, and *Trichoplusia ni* (WO 89/046699; Carbonell et al., (1985) *J. Virol. 56*:153; Wright (1986) *Nature 321*:718; Smith et al., (1983) *Mol. Cell. Biol. 3*:2156; and see generally, Fraser, *et al.* (1989) *In Vitro Cell. Dev. Biol. 25*:225).

Cells and cell culture media are commercially available for both direct and fusion expression of heterologous polypeptides in a baculovirus/expression system; cell culture technology is generally known to those skilled in the art. *See, eg,* Summers and Smith *supra*.

The modified insect cells may then be grown in an appropriate nutrient medium, which allows for stable maintenance of the plasmid(s) present in the modified insect host. Where the expression product gene is under inducible control, the host may be grown to high density, and expression induced. Alternatively, where expression is constitutive, the product will be continuously expressed into the medium and the nutrient medium must be continuously circulated, while removing the product of interest and augmenting depleted nutrients. The product may be purified by such techniques as chromatography, eg, HPLC, affinity chromatography, ion exchange chromatography, etc.; electrophoresis; density gradient centrifugation; solvent extraction, or the like. As appropriate, the product may be further purified, as required, so as to remove substantially any insect proteins which are also secreted in the medium or result from lysis of insect cells, so as to provide a product which is at least substantially free of host debris, eg, proteins, lipids and polysaccharides.

In order to obtain protein expression, recombinant host cells derived from the transformants are incubated under conditions which allow expression of the recombinant protein encoding sequence. These conditions will vary, dependent upon the host cell selected. However, the conditions are readily ascertainable to those of ordinary skill in the art, based upon what is known in the art.

iii. Plant Systems

There are many plant cell culture and whole plant genetic expression systems known in the art. Exemplary plant cellular genetic expression systems include those described in patents, such as:

US 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30:3861-3863 (1991). Descriptions of plant protein signal peptides may be found in addition to the references described above in Vaulcombe et al., *Mol. Gen. Genet.* 209:33-40 (1987); Chandler et al., *Plant Molecular Biology* 3:407-418 (1984); Rogers, *J. Biol. Chem.* 260:3731-3738 (1985); Rothstein et al., *Gene* 55:353-356 (1987); Whittier et al., Nucleic Acids Research 15:2515-2535 (1987); Wirsel et al., *Molecular Microbiology* 3:3-14 (1989); Yu et al., *Gene* 122:247-253 (1992). A description of the regulation of plant gene expression by the phytohormone, gibberellic acid and secreted enzymes induced by gibberellic acid can be found in R.L. Jones and J. MacMillin, Gibberellins: in: *Advanced Plant Physiology*,. Malcolm B. Wilkins, ed., 1984 Pitman Publishing Limited, London, pp. 21-52. References that describe other metabolically-regulated genes: Sheen, *Plant Cell*, 2:1027-1038(1990); Maas et al., *EMBO J.* 9:3447-3452 (1990); Benkel and Hickey, *Proc. Natl. Acad. Sci.* 84:1337-1339 (1987)

Typically, using techniques known in the art, a desired polynucleotide sequence is inserted into an expression cassette comprising genetic regulatory elements designed for operation in plants. The expression cassette is inserted into a desired expression vector with companion sequences upstream and downstream from the expression cassette suitable for expression in a plant host. The companion sequences will be of plasmid or viral origin and provide necessary characteristics to the vector to permit the vectors to move DNA from an original cloning host, such as bacteria, to the desired plant host. The basic bacterial/plant vector construct will preferably provide a broad host range prokaryote replication origin; a prokaryote selectable marker; and, for Agrobacterium transformations, T DNA sequences for Agrobacterium-mediated transfer to plant chromosomes. Where the heterologous gene is not readily amenable to detection, the construct will preferably also have a selectable marker gene suitable for determining if a plant cell has been transformed. A general review of suitable markers, for example for the members of the grass family, is found in Wilmink and Dons, 1993, *Plant Mol. Biol. Reptr*, 11(2):165-185.

Sequences suitable for permitting integration of the heterologous sequence into the plant genome are also recommended. These might include transposon sequences and the like for homologous recombination as well as Ti sequences which permit random insertion of a heterologous expression cassette into a plant genome. Suitable prokaryote selectable markers include resistance

toward antibiotics such as ampicillin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art.

The nucleic acid molecules of the subject invention may be included into an expression cassette for expression of the protein(s) of interest. Usually, there will be only one expression cassette, although two or more are feasible. The recombinant expression cassette will contain in addition to the heterologous protein encoding sequence the following elements, a promoter region, plant 5' untranslated sequences, initiation codon depending upon whether or not the structural gene comes equipped with one, and a transcription and translation termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the cassette allow for easy insertion into a pre-existing vector.

A heterologous coding sequence may be for any protein relating to the present invention. The sequence encoding the protein of interest will encode a signal peptide which allows processing and translocation of the protein, as appropriate, and will usually lack any sequence which might result in the binding of the desired protein of the invention to a membrane. Since, for the most part, the transcriptional initiation region will be for a gene which is expressed and translocated during germination, by employing the signal peptide which provides for translocation, one may also provide for translocation of the protein of interest. In this way, the protein(s) of interest will be translocated from the cells in which they are expressed and may be efficiently harvested. Typically secretion in seeds are across the aleurone or scutellar epithelium layer into the endosperm of the seed. While it is not required that the protein be secreted from the cells in which the protein is produced, this facilitates the isolation and purification of the recombinant protein.

Since the ultimate expression of the desired gene product will be in a eucaryotic cell it is desirable to determine whether any portion of the cloned gene contains sequences which will be processed out as introns by the host's spliceosome machinery. If so, site-directed mutagenesis of the "intron" region may be conducted to prevent losing a portion of the genetic message as a false intron code, Reed and Maniatis, *Cell* 41:95-105, 1985.

The vector can be microinjected directly into plant cells by use of micropipettes to mechanically transfer the recombinant DNA. Crossway, *Mol. Gen. Genet*, 202:179-185, 1985. The genetic material may also be transferred into the plant cell by using polyethylene glycol, Krens, et al.,

Nature, 296, 72-74, 1982.. Another method of introduction of nucleic acid segments is high velocity ballistic penetration by small particles with the nucleic acid either within the matrix of small beads or particles, or on the surface, Klein, et al., *Nature*, 327, 70-73, 1987 and Knudsen and Muller, 1991, *Planta*, 185:330-336 teaching particle bombardment of barley endosperm to create transgenic barley. Yet another method of introduction would be fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies, Fraley, et al., *Proc. Natl. Acad. Sci. USA*, 79, 1859-1863, 1982.

The vector may also be introduced into the plant cells by electroporation. (Fromm et al., *Proc. Natl Acad. Sci. USA* 82:5824, 1985). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the gene construct. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and form plant callus.

All plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be transformed by the present invention so that whole plants are recovered which contain the transferred gene. It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugarcane, sugar beet, cotton, fruit and other trees, legumes and vegetables. Some suitable plants include, for example, species from the genera *Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersion, Nicotiana, Solanum, Petunia, Digitalis, Majorana, Cichorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Hererocallis, Nemesia, Pelargonium, Panicum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Lolium, Zea, Triticum, Sorghum*, and *Datura*.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts containing copies of the heterologous gene is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced from the protoplast suspension. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Shoots and roots normally develop

simultaneously. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is fully reproducible and repeatable.

In some plant cell culture systems, the desired protein of the invention may be excreted or alternatively, the protein may be extracted from the whole plant. Where the desired protein of the invention is secreted into the medium, it may be collected. Alternatively, the embryos and embryoless-half seeds or other plant tissue may be mechanically disrupted to release any secreted protein between cells and tissues. The mixture may be suspended in a buffer solution to retrieve soluble proteins. Conventional protein isolation and purification methods will be then used to purify the recombinant protein. Parameters of time, temperature pH, oxygen, and volumes will be adjusted through routine methods to optimize expression and recovery of heterologous protein.

iv. Bacterial Systems

Bacterial expression techniques are known in the art. A bacterial promoter is any DNA sequence capable of binding bacterial RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site and a transcription initiation site. A bacterial promoter may also have a second domain called an operator, that may overlap an adjacent RNA polymerase binding site at which RNA synthesis begins. The operator permits negative regulated (inducible) transcription, as a gene repressor protein may bind the operator and thereby inhibit transcription of a specific gene. Constitutive expression may occur in the absence of negative regulatory elements, such as the operator. In addition, positive regulation may be achieved by a gene activator protein binding sequence, which, if present is usually proximal (5') to the RNA polymerase binding sequence. An example of a gene activator protein is the catabolite activator protein (CAP), which helps initiate transcription of the lac operon in Escherichia coli (E. coli) [Raibaud *et al.* (1984) *Annu. Rev. Genet. 18*:173]. Regulated expression may therefore be either positive or negative, thereby either enhancing or reducing transcription.

Sequences encoding metabolic pathway enzymes provide particularly useful promoter sequences. Examples include promoter sequences derived from sugar metabolizing enzymes, such as galactose, lactose (*lac*) [Chang *et al.* (1977) *Nature 198*:1056], and maltose. Additional examples

include promoter sequences derived from biosynthetic enzymes such as tryptophan (*trp*) [Goeddel *et al.* (1980) *Nuc. Acids Res.* 8:4057; Yelverton *et al.* (1981) *Nucl. Acids Res.* 9:731; US patent 4,738,921; EP-A-0036776 and EP-A-0121775]. The g-laotamase (*bla*) promoter system [Weissmann (1981) "The cloning of interferon and other mistakes." In *Interferon 3* (ed. I. Gresser)], bacteriophage lambda PL [Shimatake *et al.* (1981) *Nature 292*:128] and T5 [US patent 4,689,406] promoter systems also provide useful promoter sequences.

In addition, synthetic promoters which do not occur in nature also function as bacterial promoters. For example, transcription activation sequences of one bacterial or bacteriophage promoter may be joined with the operon sequences of another bacterial or bacteriophage promoter, creating a synthetic hybrid promoter [US patent 4,551,433]. For example, the *tac* promoter is a hybrid *trp-lac* promoter comprised of both *trp* promoter and *lac* operon sequences that is regulated by the *lac* repressor [Amann *et al.* (1983) *Gene 25*:167; de Boer *et al.* (1983) *Proc. Natl. Acad. Sci. 80*:21]. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. A naturally occurring promoter of non-bacterial origin can also be coupled with a compatible RNA polymerase to produce high levels of expression of some genes in prokaryotes. The bacteriophage T7 RNA polymerase/promoter system is an example of a coupled promoter system [Studier *et al.* (1986) *J. Mol. Biol. 189*:113; Tabor *et al.* (1985) *Proc Natl. Acad. Sci. 82*:1074]. In addition, a hybrid promoter can also be comprised of a bacteriophage promoter and an *E. coli* operator region (EPO-A-0 267 851).

In addition to a functioning promoter sequence, an efficient ribosome binding site is also useful for the expression of foreign genes in prokaryotes. In *E. coli*, the ribosome binding site is called the Shine-Dalgarno (SD) sequence and includes an initiation codon (ATG) and a sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon [Shine *et al.* (1975) *Nature 254*:34]. The SD sequence is thought to promote binding of mRNA to the ribosome by the pairing of bases between the SD sequence and the 3' and of *E. coli* 16S rRNA [Steitz *et al.* (1979) "Genetic signals and nucleotide sequences in messenger RNA." In *Biological Regulation and Development: Gene Expression* (ed. R.F. Goldberger)]. To express eukaryotic genes and prokaryotic genes with weak ribosome-binding site [Sambrook *et al.* (1989) "Expression of cloned genes in Escherichia coli." In *Molecular Cloning: A Laboratory Manual*].

A DNA molecule may be expressed intracellularly. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide or by either *in vivo* on *in vitro* incubation with a bacterial methionine N-terminal peptidase (EPO-A-0 219 237).

Fusion proteins provide an alternative to direct expression. Usually, a DNA sequence encoding the N-terminal portion of an endogenous bacterial protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the bacteriophage lambda cell gene can be linked at the 5' terminus of a foreign gene and expressed in bacteria. The resulting fusion protein preferably retains a site for a processing enzyme (factor Xa) to cleave the bacteriophage protein from the foreign gene [Nagai *et al.* (1984) *Nature 309*:810]. Fusion proteins can also be made with sequences from the *lacZ* [Jia *et al.* (1987) *Gene 60*:197], *trpE* [Allen et al. (1987) J. Biotechnol. 5:93; Makoff *et al.* (1989) *J. Gen. Microbiol. 135*:11], and *Chey* [EP-A-0 324 647] genes. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*eg.* ubiquitin specific processing-protease) to cleave the ubiquitin from the foreign protein. Through this method, native foreign protein can be isolated [Miller *et al.* (1989) *Bio/Technology 7*:698].

Alternatively, foreign proteins can also be secreted from the cell by creating chimeric DNA molecules that encode a fusion protein comprised of a signal peptide sequence fragment that provides for secretion of the foreign protein in bacteria [US patent 4,336,336]. The signal sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). Preferably there are processing sites, which can be cleaved either *in vivo* or *in vitro* encoded between the signal peptide fragment and the foreign gene.

DNA encoding suitable signal sequences can be derived from genes for secreted bacterial proteins, such as the *E. coli* outer membrane protein gene (*ompA*) [Masui *et al.* (1983), in: *Experimental Manipulation of Gene Expression*; Ghrayeb *et al.* (1984) *EMBO J. 3*:2437] and the *E. coli* alkaline

phosphatase signal sequence (*phoA*) [Oka *et al.* (1985) *Proc. Natl. Acad. Sci. 82*:7212]. As an additional example, the signal sequence of the alpha-amylase gene from various Bacillus strains can be used to secrete heterologous proteins from *B. subtilis* [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 244 042].

Usually, transcription termination sequences recognized by bacteria are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Transcription termination sequences frequently include DNA sequences of about 50 nucleotides capable of forming stem loop structures that aid in terminating transcription. Examples include transcription termination sequences derived from genes with strong promoters, such as the *trp* gene in *E. coli* as well as other biosynthetic genes.

Usually, the above described components, comprising a promoter, signal sequence (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg.* plasmids) capable of stable maintenance in a host, such as bacteria. The replicon will have a replication system, thus allowing it to be maintained in a prokaryotic host either for expression or for cloning and amplification. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and usually about 10 to about 150. A host containing a high copy number plasmid will preferably contain at least about 10, and more preferably at least about 20 plasmids. Either a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host.

Alternatively, the expression constructs can be integrated into the bacterial genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to the bacterial chromosome that allows the vector to integrate. Integrations appear to result from recombinations between homologous DNA in the vector and the bacterial chromosome. For example, integrating vectors constructed with DNA from various Bacillus strains integrate into the Bacillus chromosome (EP-A- 0 127 328). Integrating vectors may also be comprised of bacteriophage or transposon sequences.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of bacterial strains that have been transformed. Selectable markers can be expressed in the bacterial host and may include genes which render bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin (neomycin), and tetracycline [Davies *et al.* (1978) *Annu. Rev. Microbiol. 32*:469]. Selectable markers may also include biosynthetic genes, such as those in the histidine, tryptophan, and leucine biosynthetic pathways.

Alternatively, some of the above described components can be put together in transformation vectors. Transformation vectors are usually comprised of a selectable market that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extra-chromosomal replicons or integrating vectors, have been developed for transformation into many bacteria. For example, expression vectors have been developed for, *inter alia*, the following bacteria: Bacillus subtilis [Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541], Escherichia coli [Shimatake *et al.* (1981) *Nature 292*:128; Amann *et al.* (1985) *Gene 40*:183; Studier *et al.* (1986) *J. Mol. Biol. 189*:113; EP-A-0 036 776,EP-A-0 136 829 and EP-A-0 136 907], Streptococcus cremoris [Powell *et al.* (1988) *Appl. Environ. Microbiol. 54*:655]; Streptococcus lividans [Powell *et al.* (1988) *Appl. Environ. Microbiol. 54*:655], Streptomyces lividans [US patent 4,745,056].

Methods of introducing exogenous DNA into bacterial hosts are well-known in the art, and usually include either the transformation of bacteria treated with $CaCl_2$ or other agents, such as divalent cations and DMSO. DNA can also be introduced into bacterial cells by electroporation. Transformation procedures usually vary with the bacterial species to be transformed. See *eg.* [Masson *et al.* (1989) *FEMS Microbiol. Lett. 60*:273; Palva *et al.* (1982) *Proc. Natl. Acad. Sci. USA 79*:5582; EP-A-0 036 259 and EP-A-0 063 953; WO 84/04541, Bacillus], [Miller *et al.* (1988) *Proc. Natl. Acad. Sci. 85*:856; Wang *et al.* (1990) *J. Bacteriol. 172*:949, Campylobacter], [Cohen *et al.* (1973) *Proc. Natl. Acad. Sci. 69*:2110; Dower *et al.* (1988) *Nucleic Acids Res. 16*:6127; Kushner (1978) "An improved method for transformation of Escherichia coli with ColE1-derived plasmids. In *Genetic Engineering: Proceedings of the International Symposium on Genetic Engineering* (eds. H.W. Boyer and S. Nicosia); Mandel *et al.* (1970) *J. Mol. Biol. 53*:159; Taketo (1988) *Biochim. Biophys. Acta 949*:318; Escherichia], [Chassy *et al.* (1987) *FEMS*

Microbiol. Lett. *44*:173 Lactobacillus]; [Fiedler *et al.* (1988) *Anal. Biochem* *170*:38, Pseudomonas]; [Augustin *et al.* (1990) *FEMS Microbiol. Lett.* *66*:203, Staphylococcus], [Barany *et al.* (1980) *J. Bacteriol.* *144*:698; Harlander (1987) "Transformation of Streptococcus lactis by electroporation, in: *Streptococcal Genetics* (ed. J. Ferretti and R. Curtiss III); Perry *et al.* (1981) *Infect. Immun.* *32*:1295; Powell *et al.* (1988) *Appl. Environ. Microbiol.* *54*:655; Somkuti *et al.* (1987) *Proc. 4th Evr. Cong. Biotechnology* *1*:412, Streptococcus].

v. Yeast Expression

Yeast expression systems are also known to one of ordinary skill in the art. A yeast promoter is any DNA sequence capable of binding yeast RNA polymerase and initiating the downstream (3') transcription of a coding sequence (*eg.* structural gene) into mRNA. A promoter will have a transcription initiation region which is usually placed proximal to the 5' end of the coding sequence. This transcription initiation region usually includes an RNA polymerase binding site (the "TATA Box") and a transcription initiation site. A yeast promoter may also have a second domain called an upstream activator sequence (UAS), which, if present, is usually distal to the structural gene. The UAS permits regulated (inducible) expression. Constitutive expression occurs in the absence of a UAS. Regulated expression may be either positive or negative, thereby either enhancing or reducing transcription.

Yeast is a fermenting organism with an active metabolic pathway, therefore sequences encoding enzymes in the metabolic pathway provide particularly useful promoter sequences. Examples include alcohol dehydrogenase (ADH) (EP-A-0 284 044), enolase, glucokinase, glucose-6-phosphate isomerase, glyceraldehyde-3-phosphate-dehydrogenase (GAP or GAPDH), hexokinase, phosphofructokinase, 3-phosphoglycerate mutase, and pyruvate kinase (PyK) (EPO-A-0 329 203). The yeast *PHO5* gene, encoding acid phosphatase, also provides useful promoter sequences [Myanohara *et al.* (1983) *Proc. Natl. Acad. Sci. USA* *80*:1].

In addition, synthetic promoters which do not occur in nature also function as yeast promoters. For example, UAS sequences of one yeast promoter may be joined with the transcription activation region of another yeast promoter, creating a synthetic hybrid promoter. Examples of such hybrid promoters include the ADH regulatory sequence linked to the GAP transcription activation region (US Patent Nos. 4,876,197 and 4,880,734). Other examples of hybrid promoters include promoters which consist of the regulatory sequences of either the *ADH2*, *GAL4*, *GAL10*,

OR *PHO5* genes, combined with the transcriptional activation region of a glycolytic enzyme gene such as GAP or PyK (EP-A-0 164 556). Furthermore, a yeast promoter can include naturally occurring promoters of non-yeast origin that have the ability to bind yeast RNA polymerase and initiate transcription. Examples of such promoters include, *inter alia*, [Cohen *et al.* (1980) *Proc. Natl. Acad. Sci. USA* *77*:1078; Henikoff *et al.* (1981) *Nature* *283*:835; Hollenberg *et al.* (1981) *Curr. Topics Microbiol. Immunol.* *96*:119; Hollenberg *et al.* (1979) "The Expression of Bacterial Antibiotic Resistance Genes in the Yeast Saccharomyces cerevisiae," in: *Plasmids of Medical, Environmental and Commercial Importance* (eds. K.N. Timmis and A. Puhler); Mercerau-Puigalon *et al.* (1980) *Gene* *11*:163; Panthier *et al.* (1980) *Curr. Genet.* *2*:109;].

A DNA molecule may be expressed intracellularly in yeast. A promoter sequence may be directly linked with the DNA molecule, in which case the first amino acid at the N-terminus of the recombinant protein will always be a methionine, which is encoded by the ATG start codon. If desired, methionine at the N-terminus may be cleaved from the protein by *in vitro* incubation with cyanogen bromide.

Fusion proteins provide an alternative for yeast expression systems, as well as in mammalian, baculovirus, and bacterial expression systems. Usually, a DNA sequence encoding the N-terminal portion of an endogenous yeast protein, or other stable protein, is fused to the 5' end of heterologous coding sequences. Upon expression, this construct will provide a fusion of the two amino acid sequences. For example, the yeast or human superoxide dismutase (SOD) gene, can be linked at the 5' terminus of a foreign gene and expressed in yeast. The DNA sequence at the junction of the two amino acid sequences may or may not encode a cleavable site. See *eg.* EP-A-0 196 056. Another example is a ubiquitin fusion protein. Such a fusion protein is made with the ubiquitin region that preferably retains a site for a processing enzyme (*eg.* ubiquitin-specific processing protease) to cleave the ubiquitin from the foreign protein. Through this method, therefore, native foreign protein can be isolated (*eg.* WO88/024066).

Alternatively, foreign proteins can also be secreted from the cell into the growth media by creating chimeric DNA molecules that encode a fusion protein comprised of a leader sequence fragment that provide for secretion in yeast of the foreign protein. Preferably, there are processing sites encoded between the leader fragment and the foreign gene that can be cleaved either *in vivo* or *in*

vitro. The leader sequence fragment usually encodes a signal peptide comprised of hydrophobic amino acids which direct the secretion of the protein from the cell.

DNA encoding suitable signal sequences can be derived from genes for secreted yeast proteins, such as the yeast invertase gene (EP-A-0 012 873; JPO. 62,096,086) and the A-factor gene (US patent 4,588,684). Alternatively, leaders of non-yeast origin, such as an interferon leader, exist that also provide for secretion in yeast (EP-A-0 060 057).

A preferred class of secretion leaders are those that employ a fragment of the yeast alpha-factor gene, which contains both a "pre" signal sequence, and a "pro" region. The types of alpha-factor fragments that can be employed include the full-length pre-pro alpha factor leader (about 83 amino acid residues) as well as truncated alpha-factor leaders (usually about 25 to about 50 amino acid residues) (US Patents 4,546,083 and 4,870,008; EP-A-0 324 274). Additional leaders employing an alpha-factor leader fragment that provides for secretion include hybrid alpha-factor leaders made with a presequence of a first yeast, but a pro-region from a second yeast alphafactor. (*eg*. see WO 89/02463.)

Usually, transcription termination sequences recognized by yeast are regulatory regions located 3' to the translation stop codon, and thus together with the promoter flank the coding sequence. These sequences direct the transcription of an mRNA which can be translated into the polypeptide encoded by the DNA. Examples of transcription terminator sequence and other yeast-recognized termination sequences, such as those coding for glycolytic enzymes.

Usually, the above described components, comprising a promoter, leader (if desired), coding sequence of interest, and transcription termination sequence, are put together into expression constructs. Expression constructs are often maintained in a replicon, such as an extrachromosomal element (*eg*. plasmids) capable of stable maintenance in a host, such as yeast or bacteria. The replicon may have two replication systems, thus allowing it to be maintained, for example, in yeast for expression and in a prokaryotic host for cloning and amplification. Examples of such yeast-bacteria shuttle vectors include YEp24 [Botstein *et al*. (1979) *Gene 8*:17-24], pCI/1 [Brake *et al*. (1984) *Proc. Natl. Acad. Sci USA 81*:4642-4646], and YRp17 [Stinchcomb *et al*. (1982) *J. Mol. Biol. 158*:157]. In addition, a replicon may be either a high or low copy number plasmid. A high copy number plasmid will generally have a copy number ranging from about 5 to about 200, and

usually about 10 to about 150. A host containing a high copy number plasmid will preferably have at least about 10, and more preferably at least about 20. Enter a high or low copy number vector may be selected, depending upon the effect of the vector and the foreign protein on the host. See *eg*. Brake *et al*., *supra*.

Alternatively, the expression constructs can be integrated into the yeast genome with an integrating vector. Integrating vectors usually contain at least one sequence homologous to a yeast chromosome that allows the vector to integrate, and preferably contain two homologous sequences flanking the expression construct. Integrations appear to result from recombinations between homologous DNA in the vector and the yeast chromosome [Orr-Weaver *et al*. (1983) *Methods Enzymol. 101*:228-245]. An integrating vector may be directed to a specific locus in yeast by selecting the appropriate homologous sequence for inclusion in the vector. See Orr-Weaver *et al*., *supra*. One or more expression construct may integrate, possibly affecting levels of recombinant protein produced [Rine *et al*. (1983) *Proc. Natl. Acad. Sci. USA 80*:6750]. The chromosomal sequences included in the vector can occur either as a single segment in the vector, which results in the integration of the entire vector, or two segments homologous to adjacent segments in the chromosome and flanking the expression-construct in the vector, which can result in the stable integration of only the expression construct.

Usually, extrachromosomal and integrating expression constructs may contain selectable markers to allow for the selection of yeast strains that have been transformed. Selectable markers may include biosynthetic genes that can be expressed in the yeast host, such as *ADE2*, *HIS4*, *LEU2*, *TRP1*, and *ALG7*, and the G418 resistance gene, which confer resistance in yeast cells to tunicamycin and G418, respectively. In addition, a suitable selectable marker may also provide yeast with the ability to grow in the presence of toxic compounds, such as metal. For example, the presence of *CUP1* allows yeast to grow in the presence of copper ions [Butt *et al*. (1987) *Microbiol. Rev. 51*:351].

Alternatively, some of the above described components can be put together into transformation vectors. Transformation vectors are usually comprised of a selectable marker that is either maintained in a replicon or developed into an integrating vector, as described above.

Expression and transformation vectors, either extrachromosomal replicons or integrating vectors, have been developed for transformation into many yeasts. For example, expression vectors have been developed for, *inter alia*, the following yeasts:Candida albicans [Kurtz, *et al.* (1986) *Mol. Cell. Biol.* 6:142], Candida maltosa [Kunze, *et al.* (1985) *J. Basic Microbiol.* 25:141]. Hansenula polymorpha [Gleeson, *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302], Kluyveromyces fragilis [Das, *et al.* (1984) *J. Bacteriol.* 158:1165], Kluyveromyces lactis [De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:737; Van den Berg *et al.* (1990) *Bio/Technology* 8:135], Pichia guillerimondii [Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141], Pichia pastoris [Cregg, *et al.* (1985) *Mol. Cell. Biol.* 5:3376; US Patent Nos. 4,837,148 and 4,929,555], Saccharomyces cerevisiae [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75:1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163], Schizosaccharomyces pombe [Beach and Nurse (1981) *Nature* 300:706], and Yarrowia lipolytica [Davidow, *et al.* (1985) *Curr. Genet.* 10:380471 Gaillardin, *et al.* (1985) *Curr. Genet.* 10:49].

Methods of introducing exogenous DNA into yeast hosts are well-known in the art, and usually include either the transformation of spheroplasts or of intact yeast cells treated with alkali cations. Transformation procedures usually vary with the yeast species to be transformed. See *eg.* [Kurtz *et al.* (1986) *Mol. Cell. Biol.* 6:142; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; Candida]; [Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132:3459; Roggenkamp *et al.* (1986) *Mol. Gen. Genet.* 202:302; Hansenula]; [Das *et al.* (1984) *J. Bacteriol.* 158:1165; De Louvencourt *et al.* (1983) *J. Bacteriol.* 154:1165; Van den Berg *et al.* (1990) *Bio/Technology* 8:135; Kluyveromyces]; [Cregg *et al.* (1985) *Mol. Cell. Biol.* 5:3376; Kunze *et al.* (1985) *J. Basic Microbiol.* 25:141; US Patent Nos. 4,837,148 and 4,929,555; Pichia]; [Hinnen *et al.* (1978) *Proc. Natl. Acad. Sci. USA* 75;1929; Ito *et al.* (1983) *J. Bacteriol.* 153:163 Saccharomyces]; [Beach and Nurse (1981) *Nature* 300:706; Schizosaccharomyces]; [Davidow *et al.* (1985) *Curr. Genet.* 10:39; Gaillardin *et al.* (1985) *Curr. Genet.* 10:49; Yarrowia].

Antibodies

As used herein, the term "antibody" refers to a polypeptide or group of polypeptides composed of at least one antibody combining site. An "antibody combining site" is the three-dimensional binding space with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows a binding of the antibody with the antigen. "Antibody"

includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, humanised antibodies, altered antibodies, univalent antibodies, Fab proteins, and single domain antibodies.

Antibodies against the proteins of the invention are useful for affinity chromatography, immunoassays, and distinguishing/identifying Neisserial proteins.

Antibodies to the proteins of the invention, both polyclonal and monoclonal, may be prepared by conventional methods. In general, the protein is first used to immunize a suitable animal, preferably a mouse, rat, rabbit or goat. Rabbits and goats are preferred for the preparation of polyclonal sera due to the volume of serum obtainable, and the availability of labeled anti-rabbit and anti-goat antibodies. Immunization is generally performed by mixing or emulsifying the protein in saline, preferably in an adjuvant such as Freund's complete adjuvant, and injecting the mixture or emulsion parenterally (generally subcutaneously or intramuscularly). A dose of 50-200 µg/injection is typically sufficient. Immunization is generally boosted 2-6 weeks later with one or more injections of the protein in saline, preferably using Freund's incomplete adjuvant. One may alternatively generate antibodies by in vitro immunization using methods known in the art, which for the purposes of this invention is considered equivalent to *in vivo* immunization. Polyclonal antisera is obtained by bleeding the immunized animal into a glass or plastic container, incubating the blood at 25°C for one hour, followed by incubating at 4°C for 2-18 hours. The serum is recovered by centrifugation (*eg.* 1,000*g* for 10 minutes). About 20-50 ml per bleed may be obtained from rabbits.

Monoclonal antibodies are prepared using the standard method of Kohler & Milstein [*Nature* (1975) 256:495-96], or a modification thereof. Typically, a mouse or rat is immunized as described above. However, rather than bleeding the animal to extract serum, the spleen (and optionally several large lymph nodes) is removed and dissociated into single cells. If desired, the spleen cells may be screened (after removal of nonspecifically adherent cells) by applying a cell suspension to a plate or well coated with the protein antigen. B-cells expressing membrane-bound immunoglobulin specific for the antigen bind to the plate, and are not rinsed away with the rest of the suspension. Resulting B-cells, or all dissociated spleen cells, are then induced to fuse with myeloma cells to form hybridomas, and are cultured in a selective medium (*eg.* hypoxanthine, aminopterin, thymidine medium, "HAT"). The resulting hybridomas are plated by limiting dilution, and are assayed for the production of antibodies which bind specifically to the

immunizing antigen (and which do not bind to unrelated antigens). The selected MAb-secreting hybridomas are then cultured either *in vitro* (*eg.* in tissue culture bottles or hollow fiber reactors), or *in vivo* (as ascites in mice).

If desired, the antibodies (whether polyclonal or monoclonal) may be labeled using conventional techniques. Suitable labels include fluorophores, chromophores, radioactive atoms (particularly ^{32}P and ^{125}I), electron-dense reagents, enzymes, and ligands having specific binding partners. Enzymes are typically detected by their activity. For example, horseradish peroxidase is usually detected by its ability to convert 3,3',5,5'-tetramethylbenzidine (TMB) to a blue pigment, quantifiable with a spectrophotometer. "Specific binding partner" refers to a protein capable of binding a ligand molecule with high specificity, as for example in the case of an antigen and a monoclonal antibody specific therefor. Other specific binding partners include biotin and avidin or streptavidin, IgG and protein A, and the numerous receptor-ligand couples known in the art. It should be understood that the above description is not meant to categorize the various labels into distinct classes, as the same label may serve in several different modes. For example, ^{125}I may serve as a radioactive label or as an electron-dense reagent. HRP may serve as enzyme or as antigen for a MAb. Further, one may combine various labels for desired effect. For example, MAbs and avidin also require labels in the practice of this invention: thus, one might label a MAb with biotin, and detect its presence with avidin labeled with ^{125}I, or with an anti-biotin MAb labeled with HRP. Other permutations and possibilities will be readily apparent to those of ordinary skill in the art, and are considered as equivalents within the scope of the instant invention.

Pharmaceutical Compositions

Pharmaceutical compositions can comprise either polypeptides, antibodies, or nucleic acid of the invention. The pharmaceutical compositions will comprise a therapeutically effective amount of either polypeptides, antibodies, or polynucleotides of the claimed invention.

The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat, ameliorate, or prevent a desired disease or condition, or to exhibit a detectable therapeutic or preventative effect. The effect can be detected by, for example, chemical markers or antigen levels. Therapeutic effects also include reduction in physical symptoms, such as decreased body temperature. The precise effective amount for a subject will depend upon the subject's size and health, the nature and extent of the condition, and the therapeutics or

combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance. However, the effective amount for a given situation can be determined by routine experimentation and is within the judgement of the clinician.

For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

A pharmaceutical composition can also contain a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent, such as antibodies or a polypeptide, genes, and other therapeutic agents. The term refers to any pharmaceutical carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity. Suitable carriers may be large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Such carriers are well known to those of ordinary skill in the art.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable excipients is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. Typically, the therapeutic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. Liposomes are included within the definition of a pharmaceutically acceptable carrier.

Delivery Methods

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*eg.* see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Vaccines

Vaccines according to the invention may either be prophylactic (*ie.* to prevent infection) or therapeutic (*ie.* to treat disease after infection).

Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with "pharmaceutically acceptable carriers," which include any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable carriers are typically large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates (such as oil droplets or liposomes), and inactive virus particles. Such carriers are well known to those of ordinary skill in the art. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, *etc.* pathogens.

Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, etc; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59™ (WO 90/14837; Chapter 10 in *Vaccine design: the subunit and adjuvant approach*, eds. Powell & Newman, Plenum Press 1995), containing 5% Squalene, 0.5% Tween 80, and 0.5% Span 85 (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalane, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2%

Tween 80, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (3) saponin adjuvants, such as Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (5) cytokines, such as interleukins (*eg.* IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12, *etc.*), interferons (*eg.* gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc; and (6) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59™ are preferred.

As mentioned above, muramyl peptides include, but are not limited to, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-*sn*-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), *etc.*

The immunogenic compositions (*eg.* the immunising antigen/immunogen/polypeptide/protein/nucleic acid, pharmaceutically acceptable carrier, and adjuvant) typically will contain diluents, such as water, saline, glycerol, ethanol, etc. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles.

Typically, the immunogenic compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation also may be emulsified or encapsulated in liposomes for enhanced adjuvant effect, as discussed above under pharmaceutically acceptable carriers.

Immunogenic compositions used as vaccines comprise an immunologically effective amount of the antigenic or immunogenic polypeptides, as well as any other of the above-mentioned components, as needed. By "immunologically effective amount", it is meant that the administration of that amount to an individual, either in a single dose or as part of a series, is effective for treatment or prevention. This amount varies depending upon the health and physical condition of the individual to be treated, the taxonomic group of individual to be treated (*eg.* nonhuman primate, primate, *etc.*), the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment

of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials.

The immunogenic compositions are conventionally administered parenterally, *eg.* by injection, either subcutaneously, intramuscularly, or transdermally/transcutaneously (*eg.* WO98/20734). Additional formulations suitable for other modes of administration include oral and pulmonary formulations, suppositories, and transdermal applications. Dosage treatment may be a single dose schedule or a multiple dose schedule. The vaccine may be administered in conjunction with other immunoregulatory agents.

As an alternative to protein-based vaccines, DNA vaccination may be employed [*eg.* Robinson & Torres (1997) *Seminars in Immunology* 9:271-283; Donnelly *et al.* (1997) *Annu Rev Immunol* 15:617-648; see later herein].

<u>*Gene Delivery Vehicles*</u>

Gene therapy vehicles for delivery of constructs including a coding sequence of a therapeutic of the invention, to be delivered to the mammal for expression in the mammal, can be administered either locally or systemically. These constructs can utilize viral or non-viral vector approaches in *in vivo* or *ex vivo* modality. Expression of such coding sequence can be induced using endogenous mammalian or heterologous promoters. Expression of the coding sequence in vivo can be either constitutive or regulated.

The invention includes gene delivery vehicles capable of expressing the contemplated nucleic acid sequences. The gene delivery vehicle is preferably a viral vector and, more preferably, a retroviral, adenoviral, adeno-associated viral (AAV), herpes viral, or alphavirus vector. The viral vector can also be an astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, or togavirus viral vector. See generally, Jolly (1994) *Cancer Gene Therapy* 1:51-64; Kimura (1994) *Human Gene Therapy* 5:845-852; Connelly (1995) *Human Gene Therapy* 6:185-193; and Kaplitt (1994) *Nature Genetics* 6:148-153.

Retroviral vectors are well known in the art and we contemplate that any retroviral gene therapy vector is employable in the invention, including B, C and D type retroviruses, xenotropic retroviruses (for example, NZB-X1, NZB-X2 and NZB9-1 (see O'Neill (1985) *J. Virol.* 53:160) polytropic retroviruses

eg. MCF and MCF-MLV (see Kelly (1983) *J. Virol.* 45:291), spumaviruses and lentiviruses. See RNA Tumor Viruses, Second Edition, Cold Spring Harbor Laboratory, 1985.

Portions of the retroviral gene therapy vector may be derived from different retroviruses. For example, retrovector LTRs may be derived from a Murine Sarcoma Virus, a tRNA binding site from a Rous Sarcoma Virus, a packaging signal from a Murine Leukemia Virus, and an origin of second strand synthesis from an Avian Leukosis Virus.

These recombinant retroviral vectors may be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (see US patent 5,591,624). Retrovirus vectors can be constructed for site-specific integration into host cell DNA by incorporation of a chimeric integrase enzyme into the retroviral particle (see WO96/37626). It is preferable that the recombinant viral vector is a replication defective recombinant virus.

Packaging cell lines suitable for use with the above-described retrovirus vectors are well known in the art, are readily prepared (see WO95/30763 and WO92/05266), and can be used to create producer cell lines (also termed vector cell lines or "VCLs") for the production of recombinant vector particles. Preferably, the packaging cell lines are made from human parent cells (*eg.* HT1080 cells) or mink parent cell lines, which eliminates inactivation in human serum.

Preferred retroviruses for the construction of retroviral gene therapy vectors include Avian Leukosis Virus, Bovine Leukemia, Virus, Murine Leukemia Virus, Mink-Cell Focus-Inducing Virus, Murine Sarcoma Virus, Reticuloendotheliosis Virus and Rous Sarcoma Virus. Particularly preferred Murine Leukemia Viruses include 4070A and 1504A (Hartley and Rowe (1976) *J Virol* 19:19-25), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi, Gross (ATCC Nol VR-590), Kirsten, Harvey Sarcoma Virus and Rauscher (ATCC No. VR-998) and Moloney Murine Leukemia Virus (ATCC No. VR-190). Such retroviruses may be obtained from depositories or collections such as the American Type Culture Collection ("ATCC") in Rockville, Maryland or isolated from known sources using commonly available techniques.

Exemplary known retroviral gene therapy vectors employable in this invention include those described in patent applications GB2200651, EP0415731, EP0345242, EP0334301, WO89/02468; WO89/05349, WO89/09271, WO90/02806, WO90/07936, WO94/03622, WO93/25698,

WO93/25234, WO93/11230, WO93/10218, WO91/02805, WO91/02825, WO95/07994, US 5,219,740, US 4,405,712, US 4,861,719, US 4,980,289, US 4,777,127, US 5,591,624. See also Vile (1993) *Cancer Res* 53:3860-3864; Vile (1993) *Cancer Res* 53:962-967; Ram (1993) *Cancer Res* 53 (1993) 83-88; Takamiya (1992) *J Neurosci Res* 33:493-503; Baba (1993) *J Neurosurg* 79:729-735; Mann (1983) *Cell* 33:153; Cane (1984) *Proc Natl Acad Sci* 81:6349; and Miller (1990) *Human Gene Therapy* 1.

Human adenoviral gene therapy vectors are also known in the art and employable in this invention. See, for example, Berkner (1988) *Biotechniques* 6:616 and Rosenfeld (1991) *Science* 252:431, and WO93/07283, WO93/06223, and WO93/07282. Exemplary known adenoviral gene therapy vectors employable in this invention include those described in the above referenced documents and in WO94/12649, WO93/03769, WO93/19191, WO94/28938, WO95/11984, WO95/00655, WO95/27071, WO95/29993, WO95/34671, WO96/05320, WO94/08026, WO94/11506, WO93/06223, WO94/24299, WO95/14102, WO95/24297, WO95/02697, WO94/28152, WO94/24299, WO95/09241, WO95/25807, WO95/05835, WO94/18922 and WO95/09654. Alternatively, administration of DNA linked to killed adenovirus as described in Curiel (1992) *Hum. Gene Ther.* 3:147-154 may be employed. The gene delivery vehicles of the invention also include adenovirus associated virus (AAV) vectors. Leading and preferred examples of such vectors for use in this invention are the AAV-2 based vectors disclosed in Srivastava, WO93/09239. Most preferred AAV vectors comprise the two AAV inverted terminal repeats in which the native D-sequences are modified by substitution of nucleotides, such that at least 5 native nucleotides and up to 18 native nucleotides, preferably at least 10 native nucleotides up to 18 native nucleotides, most preferably 10 native nucleotides are retained and the remaining nucleotides of the D-sequence are deleted or replaced with non-native nucleotides. The native D-sequences of the AAV inverted terminal repeats are sequences of 20 consecutive nucleotides in each AAV inverted terminal repeat (*ie.* there is one sequence at each end) which are not involved in HP formation. The non-native replacement nucleotide may be any nucleotide other than the nucleotide found in the native D-sequence in the same position. Other employable exemplary AAV vectors are pWP-19, pWN-1, both of which are disclosed in Nahreini (1993) *Gene* 124:257-262. Another example of such an AAV vector is psub201 (see Samulski (1987) *J. Virol.* 61:3096). Another exemplary AAV vector is the Double-D ITR vector. Construction of the Double-D ITR vector is disclosed in US Patent 5,478,745. Still other vectors are those disclosed

in Carter US Patent 4,797,368 and Muzyczka US Patent 5,139,941, Chartejee US Patent 5,474,935, and Kotin WO94/288157. Yet a further example of an AAV vector employable in this invention is SSV9AFABTKneo, which contains the AFP enhancer and albumin promoter and directs expression predominantly in the liver. Its structure and construction are disclosed in Su (1996) *Human Gene Therapy* 7:463-470. Additional AAV gene therapy vectors are described in US 5,354,678, US 5,173,414, US 5,139,941, and US 5,252,479.

The gene therapy vectors of the invention also include herpes vectors. Leading and preferred examples are herpes simplex virus vectors containing a sequence encoding a thymidine kinase polypeptide such as those disclosed in US 5,288,641 and EP0176170 (Roizman). Additional exemplary herpes simplex virus vectors include HFEM/ICP6-LacZ disclosed in WO95/04139 (Wistar Institute), pHSVlac described in Geller (1988) *Science* 241:1667-1669 and in WO90/09441 and WO92/07945, HSV Us3::pgC-lacZ described in Fink (1992) *Human Gene Therapy* 3:11-19 and HSV 7134, 2 RH 105 and GAL4 described in EP 0453242 (Breakefield), and those deposited with the ATCC as accession numbers ATCC VR-977 and ATCC VR-260.

Also contemplated are alpha virus gene therapy vectors that can be employed in this invention. Preferred alpha virus vectors are Sindbis viruses vectors. Togaviruses, Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250; ATCC VR-1249; ATCC VR-532), and those described in US patents 5,091,309, 5,217,879, and WO92/10578. More particularly, those alpha virus vectors described in US Serial No. 08/405,627, filed March 15, 1995,WO94/21792, WO92/10578, WO95/07994, US 5,091,309 and US 5,217,879 are employable. Such alpha viruses may be obtained from depositories or collections such as the ATCC in Rockville, Maryland or isolated from known sources using commonly available techniques. Preferably, alphavirus vectors with reduced cytotoxicity are used (see USSN 08/679640).

DNA vector systems such as eukaryotic layered expression systems are also useful for expressing the nucleic acids of the invention. See WO95/07994 for a detailed description of eukaryotic layered expression systems. Preferably, the eukaryotic layered expression systems of the invention are derived from alphavirus vectors and most preferably from Sindbis viral vectors.

Other viral vectors suitable for use in the present invention include those derived from poliovirus, for example ATCC VR-58 and those described in Evans, Nature 339 (1989) 385 and Sabin (1973) *J. Biol. Standardization* 1:115; rhinovirus, for example ATCC VR-1110 and those described in Arnold (1990) *J Cell Biochem* L401; pox viruses such as canary pox virus or vaccinia virus, for example ATCC VR-111 and ATCC VR-2010 and those described in Fisher-Hoch (1989) *Proc Natl Acad Sci* 86:317; Flexner (1989) *Ann NY Acad Sci* 569:86, Flexner (1990) *Vaccine* 8:17; in US 4,603,112 and US 4,769,330 and WO89/01973; SV40 virus, for example ATCC VR-305 and those described in Mulligan (1979) *Nature* 277:108 and Madzak (1992) *J Gen Virol* 73:1533; influenza virus, for example ATCC VR-797 and recombinant influenza viruses made employing reverse genetics techniques as described in US 5,166,057 and in Enami (1990) *Proc Natl Acad Sci* 87:3802-3805; Enami & Palese (1991) *J Virol* 65:2711-2713 and Luytjes (1989) *Cell* 59:110, (see also McMichael (1983) *NEJ Med* 309:13, and Yap (1978) *Nature* 273:238 and *Nature* (1979) 277:108); human immunodeficiency virus as described in EP-0386882 and in Buchschacher (1992) *J. Virol.* 66:2731; measles virus, for example ATCC VR-67 and VR-1247 and those described in EP-0440219; Aura virus, for example ATCC VR-368; Bebaru virus, for example ATCC VR-600 and ATCC VR-1240; Cabassou virus, for example ATCC VR-922; Chikungunya virus, for example ATCC VR-64 and ATCC VR-1241; Fort Morgan Virus, for example ATCC VR-924; Getah virus, for example ATCC VR-369 and ATCC VR-1243; Kyzylagach virus, for example ATCC VR-927; Mayaro virus, for example ATCC VR-66; Mucambo virus, for example ATCC VR-580 and ATCC VR-1244; Ndumu virus, for example ATCC VR-371; Pixuna virus, for example ATCC VR-372 and ATCC VR-1245; Tonate virus, for example ATCC VR-925; Triniti virus, for example ATCC VR-469; Una virus, for example ATCC VR-374; Whataroa virus, for example ATCC VR-926; Y-62-33 virus, for example ATCC VR-375; O'Nyong virus, Eastern encephalitis virus, for example ATCC VR-65 and ATCC VR-1242; Western encephalitis virus, for example ATCC VR-70, ATCC VR-1251, ATCC VR-622 and ATCC VR-1252; and coronavirus, for example ATCC VR-740 and those described in Hamre (1966) *Proc Soc Exp Biol Med* 121:190.

Delivery of the compositions of this invention into cells is not limited to the above mentioned viral vectors. Other delivery methods and media may be employed such as, for example, nucleic acid expression vectors, polycationic condensed DNA linked or unlinked to killed adenovirus alone, for example see US Serial No. 08/366,787, filed December 30, 1994 and Curiel (1992) *Hum Gene Ther* 3:147-154 ligand linked DNA, for example see Wu (1989) *J Biol Chem* 264:16985-16987, eucaryotic cell delivery vehicles cells, for example see US Serial No.08/240,030, filed May 9, 1994, and US Serial No. 08/404,796, deposition of photopolymerized hydrogel materials,

hand-held gene transfer particle gun, as described in US Patent 5,149,655, ionizing radiation as described in US5,206,152 and in WO92/11033, nucleic charge neutralization or fusion with cell membranes. Additional approaches are described in Philip (1994) *Mol Cell Biol* 14:2411-2418 and in Woffendin (1994) *Proc Natl Acad Sci* 91:1581-1585.

Particle mediated gene transfer may be employed, for example see US Serial No. 60/023,867. Briefly, the sequence can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, as described in Wu & Wu (1987) *J. Biol. Chem* 262:4429-4432, insulin as described in Hucked (1990) *Biochem Pharmacol* 40:253-263, galactose as described in Plank (1992) *Bioconjugate Chem* 3:533-539, lactose or transferrin.

Naked DNA may also be employed. Exemplary naked DNA introduction methods are described in WO 90/11092 and US 5,580,859. Uptake efficiency may be improved using biodegradable latex beads. DNA coated latex beads are efficiently transported into cells after endocytosis initiation by the beads. The method may be improved further by treatment of the beads to increase hydrophobicity and thereby facilitate disruption of the endosome and release of the DNA into the cytoplasm.

Liposomes that can act as gene delivery vehicles are described in US 5,422,120, WO95/13796, WO94/23697, WO91/14445 and EP-524,968. As described in USSN. 60/023,867, on non-viral delivery, the nucleic acid sequences encoding a polypeptide can be inserted into conventional vectors that contain conventional control sequences for high level expression, and then be incubated with synthetic gene transfer molecules such as polymeric DNA-binding cations like polylysine, protamine, and albumin, linked to cell targeting ligands such as asialoorosomucoid, insulin, galactose, lactose, or transferrin. Other delivery systems include the use of liposomes to encapsulate DNA comprising the gene under the control of a variety of tissue-specific or ubiquitously-active promoters. Further non-viral delivery suitable for use includes mechanical delivery systems such as the approach described in Woffendin *et al* (1994) *Proc. Natl. Acad. Sci. USA* 91(24):11581-11585. Moreover, the coding sequence and the product of expression of such can be delivered through deposition of photopolymerized hydrogel materials. Other conventional methods for gene delivery that can be used for delivery of the coding sequence include, for

example, use of hand-held gene transfer particle gun, as described in US 5,149,655; use of ionizing radiation for activating transferred gene, as described in US 5,206,152 and WO92/11033

Exemplary liposome and polycationic gene delivery vehicles are those described in US 5,422,120 and 4,762,915; in WO 95/13796; WO94/23697; and WO91/14445; in EP-0524968; and in Stryer, Biochemistry, pages 236-240 (1975) W.H. Freeman, San Francisco; Szoka (1980) *Biochem Biophys Acta* 600:1; Bayer (1979) *Biochem Biophys Acta* 550:464; Rivnay (1987) *Meth Enzymol* 149:119; Wang (1987) *Proc Natl Acad Sci* 84:7851; Plant (1989) *Anal Biochem* 176:420.

A polynucleotide composition can comprises therapeutically effective amount of a gene therapy vehicle, as the term is defined above. For purposes of the present invention, an effective dose will be from about 0.01 mg/ kg to 50 mg/kg or 0.05 mg/kg to about 10 mg/kg of the DNA constructs in the individual to which it is administered.

Delivery Methods

Once formulated, the polynucleotide compositions of the invention can be administered (1) directly to the subject; (2) delivered *ex vivo*, to cells derived from the subject; or (3) *in vitro* for expression of recombinant proteins. The subjects to be treated can be mammals or birds. Also, human subjects can be treated.

Direct delivery of the compositions will generally be accomplished by injection, either subcutaneously, intraperitoneally, intravenously or intramuscularly or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Other modes of administration include oral and pulmonary administration, suppositories, and transdermal or transcutaneous applications (*eg.* see WO98/20734), needles, and gene guns or hyposprays. Dosage treatment may be a single dose schedule or a multiple dose schedule.

Methods for the *ex vivo* delivery and reimplantation of transformed cells into a subject are known in the art and described in *eg.* WO93/14778. Examples of cells useful in ex vivo applications include, for example, stem cells, particularly hematopoetic, lymph cells, macrophages, dendritic cells, or tumor cells.

Generally, delivery of nucleic acids for both *ex vivo* and *in vitro* applications can be accomplished by the following procedures, for example, dextran-mediated transfection, calcium phosphate

precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei, all well known in the art.

Polynucleotide and polypeptide pharmaceutical compositions

In addition to the pharmaceutically acceptable carriers and salts described above, the following additional agents can be used with polynucleotide and/or polypeptide compositions.

A.Polypeptides

One example are polypeptides which include, without limitation: asioloorosomucoid (ASOR); transferrin; asialoglycoproteins; antibodies; antibody fragments; ferritin; interleukins; interferons, granulocyte, macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), stem cell factor and erythropoietin. Viral antigens, such as envelope proteins, can also be used. Also, proteins from other invasive organisms, such as the 17 amino acid peptide from the circumsporozoite protein of plasmodium falciparum known as RII.

B.Hormones, Vitamins, etc.

Other groups that can be included are, for example: hormones, steroids, androgens, estrogens, thyroid hormone, or vitamins, folic acid.

C.Polyalkylenes, Polysaccharides, etc.

Also, polyalkylene glycol can be included with the desired polynucleotides/polypeptides. In a preferred embodiment, the polyalkylene glycol is polyethlylene glycol. In addition, mono-, di-, or polysaccharides can be included. In a preferred embodiment of this aspect, the polysaccharide is dextran or DEAE-dextran. Also, chitosan and poly(lactide-co-glycolide)

D.Lipids, and Liposomes

The desired polynucleotide/polypeptide can also be encapsulated in lipids or packaged in liposomes prior to delivery to the subject or to cells derived therefrom.

Lipid encapsulation is generally accomplished using liposomes which are able to stably bind or entrap and retain nucleic acid. The ratio of condensed polynucleotide to lipid preparation can vary but will generally be around 1:1 (mg DNA:micromoles lipid), or more of lipid. For a review of the

use of liposomes as carriers for delivery of nucleic acids, see, Hug and Sleight (1991) *Biochim. Biophys. Acta.* 1097:1-17; Straubinger (1983) *Meth. Enzymol.* 101:512-527.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner (1987) *Proc. Natl. Acad. Sci. USA* 84:7413-7416); mRNA (Malone (1989) *Proc. Natl. Acad. Sci. USA* 86:6077-6081); and purified transcription factors (Debs (1990) *J. Biol. Chem.* 265:10189-10192), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, NY. (See, also, Felgner *supra*). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boerhinger). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, *eg.* Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; WO90/11092 for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammelar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic acid complexes are prepared using methods known in the art. See *eg.* Straubinger (1983) *Meth. Immunol.* 101:512-527; Szoka (1978) *Proc. Natl. Acad. Sci. USA* 75:4194-4198; Papahadjopoulos (1975) *Biochim. Biophys. Acta* 394:483; Wilson (1979) *Cell* 17:77; Deamer & Bangham (1976) *Biochim. Biophys. Acta* 443:629; Ostro (1977) *Biochem. Biophys. Res. Commun.* 76:836; Fraley (1979) *Proc. Natl. Acad. Sci. USA* 76:3348); Enoch & Strittmatter (1979) *Proc. Natl. Acad. Sci. USA* 76:145; Fraley (1980) *J. Biol. Chem.* (1980) 255:10431; Szoka & Papahadjopoulos (1978) *Proc. Natl. Acad. Sci. USA* 75:145; and Schaefer-Ridder (1982) *Science* 215:166.

E.Lipoproteins

In addition, lipoproteins can be included with the polynucleotide/polypeptide to be delivered. Examples of lipoproteins to be utilized include: chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Also, modifications of naturally occurring lipoproteins can be used, such as acetylated LDL. These lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are including with the polynucleotide to be delivered, no other targeting ligand is included in the composition.

Naturally occurring lipoproteins comprise a lipid and a protein portion. The protein portion are known as apoproteins. At the present, apoproteins A, B, C, D, and E have been isolated and identified. At least two of these contain several proteins, designated by Roman numerals, AI, AII, AIV; CI, CII, CIII.

A lipoprotein can comprise more than one apoprotein. For example, naturally occurring chylomicrons comprises of A, B, C, and E, over time these lipoproteins lose A and acquire C and E apoproteins. VLDL comprises A, B, C, and E apoproteins, LDL comprises apoprotein B; and HDL comprises apoproteins A, C, and E.

The amino acid of these apoproteins are known and are described in, for example, Breslow (1985) Annu Rev. Biochem 54:699; Law (1986) Adv. Exp Med. Biol. 151:162; Chen (1986) J Biol Chem 261:12918; Kane (1980) Proc Natl Acad Sci USA 77:2465; and Utermann (1984) Hum Genet 65:232.

Lipoproteins contain a variety of lipids including, triglycerides, cholesterol (free and esters), and phospholipids. The composition of the lipids varies in naturally occurring lipoproteins. For example, chylomicrons comprise mainly triglycerides. A more detailed description of the lipid content of naturally occurring lipoproteins can be found, for example, in *Meth. Enzymol.* 128 (1986). The composition of the lipids are chosen to aid in conformation of the apoprotein for receptor binding activity. The composition of lipids can also be chosen to facilitate hydrophobic interaction and association with the polynucleotide binding molecule.

Naturally occurring lipoproteins can be isolated from serum by ultracentrifugation, for instance. Such methods are described in *Meth. Enzymol. (supra)*; Pitas (1980) *J. Biochem.* 255:5454-5460 and Mahey (1979) *J Clin. Invest* 64:743-750. Lipoproteins can also be produced by *in vitro* or recombinant methods by expression of the apoprotein genes in a desired host cell. See, for

example, Atkinson (1986) *Annu Rev Biophys Chem* 15:403 and Radding (1958) *Biochim Biophys Acta* 30: 443. Lipoproteins can also be purchased from commercial suppliers, such as Biomedical Technologies, Inc., Stoughton, Massachusetts, USA. Further description of lipoproteins can be found in Zuckermann *et al.* PCT/US97/14465.

F.Polycationic Agents

Polycationic agents can be included, with or without lipoprotein, in a composition with the desired polynucleotide/polypeptide to be delivered.

Polycationic agents, typically, exhibit a net positive charge at physiological relevant pH and are capable of neutralizing the electrical charge of nucleic acids to facilitate delivery to a desired location. These agents have both in vitro, ex vivo, and in vivo applications. Polycationic agents can be used to deliver nucleic acids to a living subject either intramuscularly, subcutaneously, etc.

The following are examples of useful polypeptides as polycationic agents: polylysine, polyarginine, polyornithine, and protamine. Other examples include histones, protamines, human serum albumin, DNA binding proteins, non-histone chromosomal proteins, coat proteins from DNA viruses, such as (X174, transcriptional factors also contain domains that bind DNA and therefore may be useful as nucleic aid condensing agents. Briefly, transcriptional factors such as C/CEBP, c-jun, c-fos, AP-1, AP-2, AP-3, CPF, Prot-1, Sp-1, Oct-1, Oct-2, CREP, and TFIID contain basic domains that bind DNA sequences.

Organic polycationic agents include: spermine, spermidine, and purtrescine.

The dimensions and of the physical properties of a polycationic agent can be extrapolated from the list above, to construct other polypeptide polycationic agents or to produce synthetic polycationic agents.

Synthetic polycationic agents which are useful include, for example, DEAE-dextran, polybrene. Lipofectin™, and lipofectAMINE™ are monomers that form polycationic complexes when combined with polynucleotides/polypeptides.

Immunodiagnostic Assays

Neisserial antigens of the invention can be used in immunoassays to detect antibody levels (or, conversely, anti-Neisserial antibodies can be used to detect antigen levels). Immunoassays based on well defined, recombinant antigens can be developed to replace invasive diagnostics methods. Antibodies to Neisserial proteins within biological samples, including for example, blood or serum samples, can be detected. Design of the immunoassays is subject to a great deal of variation, and a variety of these are known in the art. Protocols for the immunoassay may be based, for example, upon competition, or direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation. Most assays involve the use of labeled antibody or polypeptide; the labels may be, for example, fluorescent, chemiluminescent, radioactive, or dye molecules. Assays which amplify the signals from the probe are also known; examples of which are assays which utilize biotin and avidin, and enzyme-labeled and mediated immunoassays, such as ELISA assays.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the compositions of the invention, in suitable containers, along with the remaining reagents and materials (for example, suitable buffers, salt solutions, *etc.*) required for the conduct of the assay, as well as suitable set of assay instructions.

Nucleic Acid Hybridisation

"Hybridization" refers to the association of two nucleic acid sequences to one another by hydrogen bonding. Typically, one sequence will be fixed to a solid support and the other will be free in solution. Then, the two sequences will be placed in contact with one another under conditions that favor hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time of hybridization; agitation; agents to block the non-specific attachment of the liquid phase sequence to the solid support (Denhardt's reagent or BLOTTO); concentration of the sequences; use of compounds to increase the rate of association of sequences (dextran sulfate or polyethylene glycol); and the stringency of the washing conditions following hybridization. See Sambrook *et al.* [*supra*] Volume 2, chapter 9, pages 9.47 to 9.57.

"Stringency" refers to conditions in a hybridization reaction that favor association of very similar sequences over sequences that differ. For example, the combination of temperature and salt concentration should be chosen that is approximately 120 to 200°C below the calculated Tm of

the hybrid under study. The temperature and salt conditions can often be determined empirically in preliminary experiments in which samples of genomic DNA immobilized on filters are hybridized to the sequence of interest and then washed under conditions of different stringencies. See Sambrook *et al.* at page 9.50.

Variables to consider when performing, for example, a Southern blot are (1) the complexity of the DNA being blotted and (2) the homology between the probe and the sequences being detected. The total amount of the fragment(s) to be studied can vary a magnitude of 10, from 0.1 to 1μg for a plasmid or phage digest to 10^{-9} to 10^{-8} g for a single copy gene in a highly complex eukaryotic genome. For lower complexity polynucleotides, substantially shorter blotting, hybridization, and exposure times, a smaller amount of starting polynucleotides, and lower specific activity of probes can be used. For example, a single-copy yeast gene can be detected with an exposure time of only 1 hour starting with 1 μg of yeast DNA, blotting for two hours, and hybridizing for 4-8 hours with a probe of 10^{8} cpm/μg. For a single-copy mammalian gene a conservative approach would start with 10 μg of DNA, blot overnight, and hybridize overnight in the presence of 10% dextran sulfate using a probe of greater than 10^{8} cpm/μg, resulting in an exposure time of ~24 hours.

Several factors can affect the melting temperature (Tm) of a DNA-DNA hybrid between the probe and the fragment of interest, and consequently, the appropriate conditions for hybridization and washing. In many cases the probe is not 100% homologous to the fragment. Other commonly encountered variables include the length and total G+C content of the hybridizing sequences and the ionic strength and formamide content of the hybridization buffer. The effects of all of these factors can be approximated by a single equation:

$$Tm = 81 + 16.6(\log_{10}Ci) + 0.4[\%(G + C)] - 0.6(\%formamide) - 600/n - 1.5(\%mismatch).$$

where Ci is the salt concentration (monovalent ions) and *n* is the length of the hybrid in base pairs (slightly modified from Meinkoth & Wahl (1984) *Anal. Biochem.* 138: 267-284).

In designing a hybridization experiment, some factors affecting nucleic acid hybridization can be conveniently altered. The temperature of the hybridization and washes and the salt concentration during the washes are the simplest to adjust. As the temperature of the hybridization increases (*ie.* stringency), it becomes less likely for hybridization to occur between strands that are

nonhomologous, and as a result, background decreases. If the radiolabeled probe is not completely homologous with the immobilized fragment (as is frequently the case in gene family and interspecies hybridization experiments), the hybridization temperature must be reduced, and background will increase. The temperature of the washes affects the intensity of the hybridizing band and the degree of background in a similar manner. The stringency of the washes is also increased with decreasing salt concentrations.

In general, convenient hybridization temperatures in the presence of 50% formamide are 42°C for a probe with is 95% to 100% homologous to the target fragment, 37°C for 90% to 95% homology, and 32°C for 85% to 90% homology. For lower homologies, formamide content should be lowered and temperature adjusted accordingly, using the equation above. If the homology between the probe and the target fragment are not known, the simplest approach is to start with both hybridization and wash conditions which are nonstringent. If non-specific bands or high background are observed after autoradiography, the filter can be washed at high stringency and reexposed. If the time required for exposure makes this approach impractical, several hybridization and/or washing stringencies should be tested in parallel.

Nucleic Acid Probe Assays

Methods such as PCR, branched DNA probe assays, or blotting techniques utilizing nucleic acid probes according to the invention can determine the presence of cDNA or mRNA. A probe is said to "hybridize" with a sequence of the invention if it can form a duplex or double stranded complex, which is stable enough to be detected.

The nucleic acid probes will hybridize to the Neisserial nucleotide sequences of the invention (including both sense and antisense strands). Though many different nucleotide sequences will encode the amino acid sequence, the native Neisserial sequence is preferred because it is the actual sequence present in cells. mRNA represents a coding sequence and so a probe should be complementary to the coding sequence; single-stranded cDNA is complementary to mRNA, and so a cDNA probe should be complementary to the non-coding sequence.

The probe sequence need not be identical to the Neisserial sequence (or its complement) — some variation in the sequence and length can lead to increased assay sensitivity if the nucleic acid probe can form a duplex with target nucleotides, which can be detected. Also, the nucleic acid

probe can include additional nucleotides to stabilize the formed duplex. Additional Neisserial sequence may also be helpful as a label to detect the formed duplex. For example, a non-complementary nucleotide sequence may be attached to the 5' end of the probe, with the remainder of the probe sequence being complementary to a Neisserial sequence. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided that the probe sequence has sufficient complementarity with the a Neisserial sequence in order to hybridize therewith and thereby form a duplex which can be detected.

The exact length and sequence of the probe will depend on the hybridization conditions, such as temperature, salt condition and the like. For example, for diagnostic applications, depending on the complexity of the analyte sequence, the nucleic acid probe typically contains at least 10-20 nucleotides, preferably 15-25, and more preferably at least 30 nucleotides, although it may be shorter than this. Short primers generally require cooler temperatures to form sufficiently stable hybrid complexes with the template.

Probes may be produced by synthetic procedures, such as the triester method of Matteucci *et al.* [*J. Am. Chem. Soc.* (1981) 103:3185], or according to Urdea *et al.* [*Proc. Natl. Acad. Sci. USA* (1983) 80: 7461], or using commercially available automated oligonucleotide synthesizers.

The chemical nature of the probe can be selected according to preference. For certain applications, DNA or RNA are appropriate. For other applications, modifications may be incorporated *eg.* backbone modifications, such as phosphorothioates or methylphosphonates, can be used to increase *in vivo* half-life, alter RNA affinity, increase nuclease resistance *etc.* [*eg.* see Agrawal & Iyer (1995) *Curr Opin Biotechnol* 6:12-19; Agrawal (1996) *TIBTECH* 14:376-387]; analogues such as peptide nucleic acids may also be used [*eg.* see Corey (1997) *TIBTECH* 15:224-229; Buchardt *et al.* (1993) *TIBTECH* 11:384-386].

Alternatively, the polymerase chain reaction (PCR) is another well-known means for detecting small amounts of target nucleic acids. The assay is described in: Mullis *et al.* [*Meth. Enzymol.* (1987) 155: 335-350]; US patents 4,683,195 and 4,683,202. Two "primer" nucleotides hybridize with the target nucleic acids and are used to prime the reaction. The primers can comprise sequence that does not hybridize to the sequence of the amplification target (or its complement)

to aid with duplex stability or, for example, to incorporate a convenient restriction site. Typically, such sequence will flank the desired Neisserial sequence.

A thermostable polymerase creates copies of target nucleic acids from the primers using the original target nucleic acids as a template. After a threshold amount of target nucleic acids are generated by the polymerase, they can be detected by more traditional methods, such as Southern blots. When using the Southern blot method, the labelled probe will hybridize to the Neisserial sequence (or its complement).

Also, mRNA or cDNA can be detected by traditional blotting techniques described in Sambrook *et al* [*supra*]. mRNA, or cDNA generated from mRNA using a polymerase enzyme, can be purified and separated using gel electrophoresis. The nucleic acids on the gel are then blotted onto a solid support, such as nitrocellulose. The solid support is exposed to a labelled probe and then washed to remove any unhybridized probe. Next, the duplexes containing the labeled probe are detected. Typically, the probe is labelled with a radioactive moiety.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1-7 show biochemical data and sequence analysis pertaining to Examples 1, 2, 3, 7, 13, 16 and 19, respectively, with ORFs 40, 38, 44, 52, 114, 41 and 124.. M1 and M2 are molecular weight markers. Arrows indicate the position of the main recombinant product or, in Western blots, the position of the main *N.meningitidis* immunoreactive band. TP indicates *N.meningitidis* total protein extract; OMV indicates *N.meningitidis* outer membrane vesicle preparation. In bactericidal assay results: a diamond (◇) shows preimmune data; a triangle (△) shows GST control data; a circle (○) shows data with recombinant *N.meningitidis* protein. Computer analyses show a hydrophilicity plot (upper), an antigenic index plot (middle), and an AMPHI analysis (lower). The AMPHI program has been used to predict T-cell epitopes [Gao *et al.* (1989) *J. Immunol.* <u>143</u>:3007; Roberts *et al.* (1996) *AIDS Res Hum Retrovir* <u>12</u>:593; Quakyi *et al.* (1992) *Scand J Immunol* suppl.11:9) and is available in the Protean package of DNASTAR, Inc. (1228 South Park Street, Madison, Wisconsin 53715 USA).

EXAMPLES

The examples describe nucleic acid sequences which have been identified in *N.meningitidis*, along with their putative translation products. Not all of the nucleic acid sequences are complete *ie.* they encode less than the full-length wild-type protein. It is believed at present that none of the DNA sequences described herein have significant homologs in *N.gonorrhoeae.*

The examples are generally in the following format:

- a nucleotide sequence which has been identified in *N.meningitidis* (strain B)
- the putative translation product of this sequence
- a computer analysis of the translation product based on database comparisons
- a corresponding gene and protein sequence identified in *N.meningitidis* (strain A)
- a description of the characteristics of the proteins which indicates that they might be suitably antigenic
- results of biochemical analysis (expression, purification, ELISA, FACS *etc.*)

The examples typically include details of sequence homology between species and strains. Proteins that are similar in sequence are generally similar in both structure and function, and the homology often indicates a common evolutionary origin. Comparison with sequences of proteins of known function is widely used as a guide for the assignment of putative protein function to a new sequence and has proved particularly useful in whole-genome analyses.

Sequence comparisons were performed at NCBI (http://www.ncbi.nlm.nih.gov) using the algorithms BLAST, BLAST2, BLASTn, BLASTp, tBLASTn, BLASTx, & tBLASTx [*eg.* see also Altschul *et al.* (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25:2289-3402]. Searches were performed against the following databases: non-redundant GenBank+EMBL+DDBJ+PDB sequences and non-redundant GenBank CDS translations+PDB+SwissProt+SPupdate+PIR sequences.

Dots within nucleotide sequences (*eg.* position 288 in Example 12) represent nucleotides which have been arbitrarily introduced in order to maintain a reading frame. In the same way, double-underlined nucleotides were removed. Lower case letters (*eg.* position 589 in Example 12) represent ambiguities which arose during alignment of independent sequencing reactions (some

of the nucleotide sequences in the examples are derived from combining the results of two or more experiments).

Nucleotide sequences were scanned in all six reading frames to predict the presence of hydrophobic domains using an algorithm based on the statistical studies of Esposti *et al.* [Critical evaluation of the hydropathy of membrane proteins (1990) *Eur J Biochem* 190:207-219]. These domains represent potential transmembrane regions or hydrophobic leader sequences.

Open reading frames were predicted from fragmented nucleotide sequences using the program ORFFINDER (NCBI).

Underlined amino acid sequences indicate possible transmembrane domains or leader sequences in the ORFs, as predicted by the PSORT algorithm (http://www.psort.nibb.ac.jp). Functional domains were also predicted using the MOTIFS program (GCG Wisconsin & PROSITE).

Various tests can be used to assess the *in vivo* immunogenicity of the proteins identified in the examples. For example, the proteins can be expressed recombinantly and used to screen patient sera by immunoblot. A positive reaction between the protein and patient serum indicates that the patient has previously mounted an immune response to the protein in question *ie.* the protein is an immunogen. This method can also be used to identify immunodominant proteins.

The recombinant protein can also be conveniently used to prepare antibodies *eg.* in a mouse. These can be used for direct confirmation that a protein is located on the cell-surface. Labelled antibody (*eg.* fluorescent labelling for FACS) can be incubated with intact bacteria and the presence of label on the bacterial surface confirms the location of the protein.

In particular, the following methods (A) to (S) were used to express, purify and biochemically characterise the proteins of the invention:

A) Chromosomal DNA preparation

N.meningitidis strain 2996 was grown to exponential phase in 100ml of GC medium, harvested by centrifugation, and resuspended in 5ml buffer (20% Sucrose, 50mM Tris-HCl, 50mM EDTA, pH8). After 10 minutes incubation on ice, the bacteria were lysed by adding 10ml lysis solution (50mM NaCl, 1% Na-Sarkosyl, 50μg/ml Proteinase K), and the suspension was incubated at 37°C

for 2 hours. Two phenol extractions (equilibrated to pH 8) and one $ChCl_3$/isoamylalcohol (24:1) extraction were performed. DNA was precipitated by addition of 0.3M sodium acetate and 2 volumes ethanol, and was collected by centrifugation. The pellet was washed once with 70% ethanol and redissolved in 4ml buffer (10mM Tris-HCl, 1mM EDTA, pH 8). The DNA concentration was measured by reading the OD at 260 nm.

B) Oligonucleotide design

Synthetic oligonucleotide primers were designed on the basis of the coding sequence of each ORF, using (a) the meningococcus B sequence when available, or (b) the gonococcus/meningococcus A sequence, adapted to the codon preference usage of meningococcus as necessary. Any predicted signal peptides were omitted, by deducing the 5'-end amplification primer sequence immediately downstream from the predicted leader sequence.

The 5' primers included two restriction enzyme recognition sites (*BamHI-NdeI*, *BamHI-NheI*, or *EcoRI-NheI*, depending on the gene's own restriction pattern); the 3' primers included a *XhoI* restriction site. This procedure was established in order to direct the cloning of each amplification product (corresponding to each ORF) into two different expression systems: pGEX-KG (using either *BamHI-XhoI* or *EcoRI-XhoI*), and pET21b+ (using either *NdeI-XhoI* or *NheI-XhoI*).

5'-end primer tail:	CGC<u>GGATCC</u><u>CATATG</u>	(*BamHI-NdeI*)
	CGC<u>GGATCC</u><u>GCTAGC</u>	(*BamHI-NheI*)
	CCG<u>GAATTC</u>TA<u>GCTAGC</u>	(*EcoRI-NheI*)
3'-end primer tail:	CCCG<u>CTCGAG</u>	(*XhoI*)

As well as containing the restriction enzyme recognition sequences, the primers included nucleotides which hybridised to the sequence to be amplified. The number of hybridizing nucleotides depended on the melting temperature of the whole primer, and was determined for each primer using the formulae:

$T_m = 4\,(G+C) + 2\,(A+T)$ (tail excluded)

$T_m = 64.9 + 0.41\,(\%\,GC) - 600/N$ (whole primer)

The average melting temperature of the selected oligos were 65-70°C for the whole oligo and 50-55°C for the hybridising region alone.

Table 1 shows the forward and reverse primers used for each amplification. Oligos were synthesized by a Perkin Elmer 394 DNA/RNA Synthesizer, eluted from the columns in 2ml NH_4OH, and deprotected by 5 hours incubation at 56°C. The oligos were precipitated by addition of 0.3M Na-Acetate and 2 volumes ethanol. The samples were then centrifuged and the pellets resuspended in either 100μl or 1ml of water. OD_{260} was determined using a Perkin Elmer Lambda Bio spectrophotometer and the concentration was determined and adjusted to 2-10pmol/μl.

C) Amplification

The standard PCR protocol was as follows: 50-200ng of genomic DNA were used as a template in the presence of 20-40μM of each oligo, 400-800μM dNTPs solution, 1x PCR buffer (including 1.5mM $MgCl_2$), 2.5 units *TaqI* DNA polymerase (using Perkin-Elmer AmpliTaQ, GIBCO Platinum, Pwo DNA polymerase, or Tahara Shuzo Taq polymerase).

In some cases, PCR was optimised by the addition of 10μl DMSO or 50μl 2M betaine.

After a hot start (adding the polymerase during a preliminary 3 minute incubation of the whole mix at 95°C), each sample underwent a double-step amplification: the first 5 cycles were performed using as the hybridization temperature the one of the oligos excluding the restriction enzymes tail, followed by 30 cycles performed according to the hybridization temperature of the whole length oligos. The cycles were followed by a final 10 minute extension step at 72°C.

The standard cycles were as follows:

	Denaturation	Hybridisation	Elongation
First 5 cycles	30 seconds 95°C	30 seconds 50-55°C	30-60 seconds 72°C
Last 30 cycles	30 seconds 95°C	30 seconds 65-70°C	30-60 seconds 72°C

The elongation time varied according to the length of the ORF to be amplified.

The amplifications were performed using either a 9600 or a 2400 Perkin Elmer GeneAmp PCR System. To check the results, 1/10 of the amplification volume was loaded onto a 1-1.5% agarose gel and the size of each amplified fragment compared with a DNA molecular weight marker.

The amplified DNA was either loaded directly on a 1% agarose gel or first precipitated with ethanol and resuspended in a suitable volume to be loaded on a 1% agarose gel. The DNA fragment corresponding to the right size band was then eluted and purified from gel, using the Qiagen Gel Extraction Kit, following the instructions of the manufacturer. The final volume of the DNA fragment was 30µl or 50µl of either water or 10mM Tris, pH 8.5.

D) Digestion of PCR fragments

The purified DNA corresponding to the amplified fragment was split into 2 aliquots and double-digested with:

- *NdeI/XhoI* or *NheI/XhoI* for cloning into pET-21b+ and further expression of the protein as a C-terminus His-tag fusion

- *BamHI/XhoI* or *EcoRI/XhoI* for cloning into pGEX-KG and further expression of the protein as N-terminus GST fusion.

- *EcoRI/PstI, EcoRI/SalI, SalI/PstI* for cloning into pGex-His and further expression of the protein as N-terminus His-tag fusion

Each purified DNA fragment was incubated (37°C for 3 hours to overnight) with 20 units of each restriction enzyme (New England Biolabs) in a either 30 or 40µl final volume in the presence of the appropriate buffer. The digestion product was then purified using the QIAquick PCR purification kit, following the manufacturer's instructions, and eluted in a final volume of 30 or 50µl of either water or 10mM Tris-HCl, pH 8.5. The final DNA concentration was determined by 1% agarose gel electrophoresis in the presence of titrated molecular weight marker.

E) Digestion of the cloning vectors (pET22B, pGEX-KG, pTRC-His A, and pGex-His)

10µg plasmid was double-digested with 50 units of each restriction enzyme in 200µl reaction volume in the presence of appropriate buffer by overnight incubation at 37°C. After loading the

whole digestion on a 1% agarose gel, the band corresponding to the digested vector was purified from the gel using the Qiagen QIAquick Gel Extraction Kit and the DNA was eluted in 50µl of 10mM Tris-HCl, pH 8.5. The DNA concentration was evaluated by measuring OD_{260} of the sample, and adjusted to 50µg/µl. 1µl of plasmid was used for each cloning procedure.

The vector pGEX-His is a modified pGEX-2T vector carrying a region encoding six histidine residues upstream to the thrombin cleavage site and containing the multiple cloning site of the vector pTRC99 (Pharmacia).

F) Cloning

The fragments corresponding to each ORF, previously digested and purified, were ligated in both pET22b and pGEX-KG. In a final volume of 20µl, a molar ratio of 3:1 fragment/vector was ligated using 0.5µl of NEB T4 DNA ligase (400 units/µl), in the presence of the buffer supplied by the manufacturer. The reaction was incubated at room temperature for 3 hours. In some experiments, ligation was performed using the Boehringer "Rapid Ligation Kit", following the manufacturer's instructions.

In order to introduce the recombinant plasmid in a suitable strain, 100µl *E. coli* DH5 competent cells were incubated with the ligase reaction solution for 40 minutes on ice, then at 37°C for 3 minutes, then, after adding 800µl LB broth, again at 37°C for 20 minutes. The cells were then centrifuged at maximum speed in an Eppendorf microfuge and resuspended in approximately 200µl of the supernatant. The suspension was then plated on LB ampicillin (100mg/ml).

The screening of the recombinant clones was performed by growing 5 randomly-chosen colonies overnight at 37°C in either 2ml (pGEX or pTC clones) or 5ml (pET clones) LB broth + 100µg/ml ampicillin. The cells were then pelletted and the DNA extracted using the Qiagen QIAprep Spin Miniprep Kit, following the manufacturer's instructions, to a final volume of 30µl. 5µl of each individual miniprep (approximately 1g) were digested with either *NdeI/XhoI* or *BamHI/XhoI* and the whole digestion loaded onto a 1-1.5% agarose gel (depending on the expected insert size), in parallel with the molecular weight marker (1Kb DNA Ladder, GIBCO). The screening of the positive clones was made on the base of the correct insert size.

G) Expression

Each ORF cloned into the expression vector was transformed into the strain suitable for expression of the recombinant protein product. 1μl of each construct was used to transform 30μl of *E.coli* BL21 (pGEX vector), *E.coli* TOP 10 (pTRC vector) or *E.coli* BL21-DE3 (pET vector), as described above. In the case of the pGEX-His vector, the same *E.coli* strain (W3110) was used for initial cloning and expression. Single recombinant colonies were inoculated into 2ml LB+Amp (100μg/ml), incubated at 37°C overnight, then diluted 1:30 in 20ml of LB+Amp (100μg/ml) in 100ml flasks, making sure that the OD_{600} ranged between 0.1 and 0.15. The flasks were incubated at 30°C into gyratory water bath shakers until OD indicated exponential growth suitable for induction of expression (0.4-0.8 OD for pET and pTRC vectors; 0.8-1 OD for pGEX and pGEX-His vectors). For the pET, pTRC and pGEX-His vectors, the protein expression was induced by addition of 1mM IPTG, whereas in the case of pGEX system the final concentration of IPTG was 0.2mM. After 3 hours incubation at 30°C, the final concentration of the sample was checked by OD. In order to check expression, 1ml of each sample was removed, centrifuged in a microfuge, the pellet resuspended in PBS, and analysed by 12% SDS-PAGE with Coomassie Blue staining. The whole sample was centrifuged at 6000*g* and the pellet resuspended in PBS for further use.

H) GST-fusion proteins large-scale purification.

A single colony was grown overnight at 37°C on LB+Amp agar plate. The bacteria were inoculated into 20ml of LB+Amp liquid culture in a water bath shaker and grown overnight. Bacteria were diluted 1:30 into 600ml of fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD_{550} 0.8-1. Protein expression was induced with 0.2mM IPTG followed by three hours incubation. The culture was centrifuged at 8000rpm at 4°C. The supernatant was discarded and the bacterial pellet was resuspended in 7.5ml cold PBS. The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed twice and centrifuged again. The supernatant was collected and mixed with 150μl Glutatione-Sepharose 4B resin (Pharmacia) (previously washed with PBS) and incubated at room temperature for 30 minutes. The sample was centrifuged at 700*g* for 5 minutes at 4°C. The resin was washed twice with 10ml cold PBS for 10 minutes, resuspended in 1ml cold PBS, and loaded on a disposable column. The resin was washed twice with 2ml cold PBS until the flow-through reached OD_{280} of 0.02-0.06. The GST-fusion protein was eluted by addition of 700μl cold Glutathione elution buffer

(10mM reduced glutathione, 50mM Tris-HCl) and fractions collected until the OD_{280} was 0.1. 21μl of each fraction were loaded on a 12% SDS gel using either Biorad SDS-PAGE Molecular weight standard broad range (M1) (200, 116.25, 97.4, 66.2, 45, 31, 21.5, 14.4, 6.5 kDa) or Amersham Rainbow Marker (M2) (220, 66, 46, 30, 21.5, 14.3 kDa) as standards. As the MW of GST is 26kDa, this value must be added to the MW of each GST-fusion protein.

I) His-fusion solubility analysis

To analyse the solubility of the His-fusion expression products, pellets of 3ml cultures were resuspended in buffer M1 [500μl PBS pH 7.2]. 25μl lysozyme (10mg/ml) was added and the bacteria were incubated for 15 min at 4°C. The pellets were sonicated for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed twice and then separated again into pellet and supernatant by a centrifugation step. The supernatant was collected and the pellet was resuspended in buffer M2 [8M urea, 0.5M NaCl, 20mM imidazole and 0.1M NaH_2PO_4] and incubated for 3 to 4 hours at 4°C. After centrifugation, the supernatant was collected and the pellet was resuspended in buffer M3 [6M guanidinium-HCl, 0.5M NaCl, 20mM imidazole and 0.1M NaH_2PO_4] overnight at 4°C. The supernatants from all steps were analysed by SDS-PAGE.

J) His-fusion large-scale purification.

A single colony was grown overnight at 37°C on a LB + Amp agar plate. The bacteria were inoculated into 20ml of LB+Amp liquid culture and incubated overnight in a water bath shaker. Bacteria were diluted 1:30 into 600ml fresh medium and allowed to grow at the optimal temperature (20-37°C) to OD_{550} 0.6-0.8. Protein expression was induced by addition of 1mM IPTG and the culture further incubated for three hours. The culture was centrifuged at 8000rpm at 4°C, the supernatant was discarded and the bacterial pellet was resuspended in 7.5ml of either (i) cold buffer A (300mM NaCl, 50mM phosphate buffer, 10mM imidazole, pH 8) for soluble proteins or (ii) buffer B (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 8.8) for insoluble proteins.

The cells were disrupted by sonication on ice for 30 sec at 40W using a Branson sonifier B-15, frozen and thawed two times and centrifuged again.

For insoluble proteins, the supernatant was stored at -20°C, while the pellets were resuspended in 2ml buffer C (6M guanidine hydrochloride, 100mM phosphate buffer, 10mM Tris-HCl, pH 7.5) and treated in a homogenizer for 10 cycles. The product was centrifuged at 13000rpm for 40 minutes.

Supernatants were collected and mixed with 150μl Ni^{2+}-resin (Pharmacia) (previously washed with either buffer A or buffer B, as appropriate) and incubated at room temperature with gentle agitation for 30 minutes. The sample was centrifuged at 700*g* for 5 minutes at 4°C. The resin was washed twice with 10ml buffer A or B for 10 minutes, resuspended in 1ml buffer A or B and loaded on a disposable column. The resin was washed at either (i) 4°C with 2ml cold buffer A or (ii) room temperature with 2ml buffer B, until the flow-through reached OD_{280} of 0.02-0.06.

The resin was washed with either (i) 2ml cold 20mM imidazole buffer (300mM NaCl, 50mM phosphate buffer, 20mM imidazole, pH 8) or (ii) buffer D (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 6.3) until the flow-through reached the $O.D_{280}$ of 0.02-0.06. The His-fusion protein was eluted by addition of 700μl of either (i) cold elution buffer A (300mM NaCl, 50mM phosphate buffer, 250mM imidazole, pH 8) or (ii) elution buffer B (urea 8M, 10mM Tris-HCl, 100mM phosphate buffer, pH 4.5) and fractions collected until the $O.D_{280}$ was 0.1. 21μl of each fraction were loaded on a 12% SDS gel.

K) His-fusion proteins renaturation

10% glycerol was added to the denatured proteins. The proteins were then diluted to 20μg/ml using dialysis buffer I (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, 2M urea, pH 8.8) and dialysed against the same buffer at 4°C for 12-14 hours. The protein was further dialysed against dialysis buffer II (10% glycerol, 0.5M arginine, 50mM phosphate buffer, 5mM reduced glutathione, 0.5mM oxidised glutathione, pH 8.8) for 12-14 hours at 4°C. Protein concentration was evaluated using the formula:

$$\text{Protein (mg/ml)} = (1.55 \times OD_{280}) - (0.76 \times OD_{260})$$

L) His-fusion large-scale purification

500ml of bacterial cultures were induced and the fusion proteins were obtained soluble in buffer M1, M2 or M3 using the procedure described above. The crude extract of the bacteria was loaded

onto a Ni-NTA superflow column (Qiagen) equilibrated with buffer M1, M2 or M3 depending on the solubilization buffer of the fusion proteins. Unbound material was eluted by washing the column with the same buffer. The specific protein was eluted with the corresponding buffer containing 500mM imidazole and dialysed against the corresponding buffer without imidazole. After each run the columns were sanitized by washing with at least two column volumes of 0.5 M sodium hydroxide and reequilibrated before the next use.

M) Mice immunisations

20μg of each purified protein were used to immunise mice intraperitoneally. In the case of ORF 44, CD1 mice were immunised with $Al(OH)_3$ as adjuvant on days 1, 21 and 42, and immune response was monitored in samples taken on day 56. For ORF 40, CD1 mice were immunised using Freund's adjuvant, rather than $Al(OH)_3$, and the same immunisation protocol was used, except that the immune response was measured on day 42, rather than 56. Similarly, for ORF 38, CD1 mice were immunised with Freund's adjuvant, but the immune response was measured on day 49.

N) ELISA assay (sera analysis)

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 7ml of Mueller-Hinton Broth (Difco) containing 0.25% Glucose. Bacterial growth was monitored every 30 minutes by following OD_{620}. The bacteria were let to grow until the OD reached the value of 0.3-0.4. The culture was centrifuged for 10 minutes at 10000rpm. The supernatant was discarded and bacteria were washed once with PBS, resuspended in PBS containing 0.025% formaldehyde, and incubated for 2 hours at room temperature and then overnight at 4°C with stirring. 100μl bacterial cells were added to each well of a 96 well Greiner plate and incubated overnight at 4°C. The wells were then washed three times with PBT washing buffer (0.1% Tween-20 in PBS). 200μl of saturation buffer (2.7% Polyvinylpyrrolidone 10 in water) was added to each well and the plates incubated for 2 hours at 37°C. Wells were washed three times with PBT. 200μl of diluted sera (Dilution buffer: 1% BSA, 0.1% Tween-20, 0.1% NaN_3 in PBS) were added to each well and the plates incubated for 90 minutes at 37°C. Wells were washed three times with PBT. 100μl of HRP-conjugated rabbit anti-mouse (Dako) serum

diluted 1:2000 in dilution buffer were added to each well and the plates were incubated for 90 minutes at 37°C. Wells were washed three times with PBT buffer. 100μl of substrate buffer for HRP (25ml of citrate buffer pH5, 10mg of O-phenildiamine and 10μl of H_2O) were added to each well and the plates were left at room temperature for 20 minutes. 100μl H_2SO_4 was added to each well and OD_{490} was followed. The ELISA was considered positive when OD_{490} was 2.5 times the respective pre-immune sera.

O) FACScan bacteria Binding Assay procedure.

The acapsulated MenB M7 strain was plated on chocolate agar plates and incubated overnight at 37°C. Bacterial colonies were collected from the agar plates using a sterile dracon swab and inoculated into 4 tubes containing 8ml each Mueller-Hinton Broth (Difco) containing 0.25% glucose. Bacterial growth was monitored every 30 minutes by following OD_{620}. The bacteria were let to grow until the OD reached the value of 0.35-0.5. The culture was centrifuged for 10 minutes at 4000rpm. The supernatant was discarded and the pellet was resuspended in blocking buffer (1% BSA, 0.4% NaN_3) and centrifuged for 5 minutes at 4000rpm. Cells were resuspended in blocking buffer to reach OD_{620} of 0.07. 100μl bacterial cells were added to each well of a Costar 96 well plate. 100μl of diluted (1:200) sera (in blocking buffer) were added to each well and plates incubated for 2 hours at 4°C. Cells were centrifuged for 5 minutes at 4000rpm, the supernatant aspirated and cells washed by addition of 200μl/well of blocking buffer in each well. 100μl of R-Phicoerytrin conjugated $F(ab)_2$ goat anti-mouse, diluted 1:100, was added to each well and plates incubated for 1 hour at 4°C. Cells were spun down by centrifugation at 4000rpm for 5 minutes and washed by addition of 200μl/well of blocking buffer. The supernatant was aspirated and cells resuspended in 200μl/well of PBS, 0.25% formaldehyde. Samples were transferred to FACScan tubes and read. The condition for FACScan setting were: FL1 on, FL2 and FL3 off; FSC-H threshold:92; FSC PMT Voltage: E 02; SSC PMT: 474; Amp. Gains 7.1; FL-2 PMT: 539; compensation values: 0.

P) OMV preparations

Bacteria were grown overnight on 5 GC plates, harvested with a loop and resuspended in 10 ml 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes and the bacteria disrupted by sonication for 10 minutes on ice (50% duty cycle, 50% output). Unbroken cells were removed by

centrifugation at 5000*g* for 10 minutes and the total cell envelope fraction recovered by centrifugation at 50000*g* at 4°C for 75 minutes. To extract cytoplasmic membrane proteins from the crude outer membranes, the whole fraction was resuspended in 2% sarkosyl (Sigma) and incubated at room temperature for 20 minutes. The suspension was centrifuged at 10000*g* for 10 minutes to remove aggregates, and the supernatant further ultracentrifuged at 50000*g* for 75 minutes to pellet the outer membranes. The outer membranes were resuspended in 10mM Tris-HCl, pH8 and the protein concentration measured by the Bio-Rad Protein assay, using BSA as a standard.

Q) Whole Extracts preparation

Bacteria were grown overnight on a GC plate, harvested with a loop and resuspended in 1ml of 20mM Tris-HCl. Heat inactivation was performed at 56°C for 30 minutes.

R) Western blotting

Purified proteins (500ng/lane), outer membrane vesicles (5μg) and total cell extracts (25μg) derived from MenB strain 2996 were loaded on 15% SDS-PAGE and transferred to a nitrocellulose membrane. The transfer was performed for 2 hours at 150mA at 4°C, in transferring buffer (0.3 % Tris base, 1.44 % glycine, 20% methanol). The membrane was saturated by overnight incubation at 4°C in saturation buffer (10% skimmed milk, 0.1% Triton X100 in PBS). The membrane was washed twice with washing buffer (3% skimmed milk, 0.1% Triton X100 in PBS) and incubated for 2 hours at 37°C with mice sera diluted 1:200 in washing buffer. The membrane was washed twice and incubated for 90 minutes with a 1:2000 dilution of horseradish peroxidase labelled anti-mouse Ig. The membrane was washed twice with 0.1% Triton X100 in PBS and developed with the Opti-4CN Substrate Kit (Bio-Rad). The reaction was stopped by adding water.

S) Bactericidal assay

MC58 strain was grown overnight at 37°C on chocolate agar plates. 5-7 colonies were collected and used to inoculate 7ml Mueller-Hinton broth. The suspension was incubated at 37°C on a nutator and let to grow until OD_{620} was 0.5-0.8. The culture was aliquoted into sterile 1.5ml Eppendorf tubes and centrifuged for 20 minutes at maximum speed in a microfuge. The pellet was

washed once in Gey's buffer (Gibco) and resuspended in the same buffer to an OD_{620} of 0.5, diluted 1:20000 in Gey's buffer and stored at 25°C.

50µl of Gey's buffer/1% BSA was added to each well of a 96-well tissue culture plate. 25µl of diluted mice sera (1:100 in Gey's buffer/0.2% BSA) were added to each well and the plate incubated at 4°C. 25µl of the previously described bacterial suspension were added to each well. 25µl of either heat-inactivated (56°C waterbath for 30 minutes) or normal baby rabbit complement were added to each well. Immediately after the addition of the baby rabbit complement, 22µl of each sample/well were plated on Mueller-Hinton agar plates (time 0). The 96-well plate was incubated for 1 hour at 37°C with rotation and then 22µl of each sample/well were plated on Mueller-Hinton agar plates (time 1). After overnight incubation the colonies corresponding to time 0 and time 1 hour were counted.

Table II gives a summary of the cloning, expression and purification results.

Example 1

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 1>:

```
  1   ..ACACTGTTGT TTGCAACGGT TCAGGCAAGT GCTAACCAAT GAAGAGCAAG
 51     AAGAAGATTT ATATTTAGAC CCCGTACAAC GCACTGTTGC CGTGTTGATA
101     GTCAATTCCG ATAAAGAAGG CACGGGAGAA AAAGAAAAAG TAGAAGAAAA
151     TTCAGATTGG GCAGTATATT TCAACGAGAA AGGAGTACTA ACAGCCAGAG
201     AAATCACCyT CAAAGCCGGC GACAACCTGA AAATCAAACA AAACGGCACA
251     AACTTCACCT ACTCGCTGAA AAAAGACCTC AcAGATCTGA CCAGTGTTGG
301     AACTGAAAAA TTATCGTTTA GCGCAAACGG CAATAAAGTC AACATcACAA
351     GCGACACCAA AGGCTTGAAT TTTGCGAAAG AAACGGCTGG aACGAACGgC
401     GACACCACGG TTCATCTGAA CGGTATTGGT TCGACTTTGA CCGATACGCT
451     GCTGAATACC GGAGCGACCA CAAACGTAAC CAACGACAAC GTTACCGATG
501     ACGAGAAAAA ACGTGCGGCA AGCGTTAAAG ACGTATTAAA CGCTGGCTGG
551     AACATTAAAG GCGTTAAACC CGGTACAACA GCTTCCGATA ACGTTGATTT
601     CGTCCGCACT TACGACACAG TCGAGTTCTT GAGCGCAGAT ACGAAAACAA
651     CGACTGTTAA TGTGGAAAGC AAAGACAACG GCAAGAAAAC CGAAGTTAAA
701     ATCGGTGCGA AGACTTCTGT TATTAAAGAA AAAGAC...
```

This corresponds to the amino acid sequence <SEQ ID 2; ORF40>:

```
  1   ..TLLFATVQAS ANQEEQEEDL YLDPVQRTVA VLIVNSDKEG TGEKEKVEEN
 51     SDWAVYFNEK GVLTAREITX KAGDNLKIKQ NGTNFTYSLK KDLTDLTSVG
101     TEKLSFSANG NKVNITSDTK GLNFAKETAG TNGDTTVHLN GIGSTLTDTL
151     LNTGATTNVT NDNVTDDEKK RAASVKDVLN AGWNIKGVKP GTTASDNVDF
201     VRTYDTVEFL SADTKTTTVN VESKDNGKKT EVKIGAKTSV IKEKD...
```

Further work revealed the complete DNA sequence <SEQ ID 3>:

```
  1   ATGAACAAAA TATACCGCAT CATTTGGAAT AGTGCCCTCA ATGCCTGGGT
 51   CGTCGTATCC GAGCTCACAC GCAACCACAC CAAACGCGCC TCCGCAACCG
101   TGAAGACCGC CGTATTGGCG ACACTGTTGT TTGCAACGGT TCAGGCAAGT
151   GCTAACAATG AAGAGCAAGA AGAAGATTTA TATTTAGACC CCGTACAACG
```



```
 201   CACTGTTGCC GTGTTGATAG TCAATTCCGA TAAAGAAGGC ACGGGAGAAA
 251   AAGAAAAAGT AGAAGAAAAT TCAGATTGGG CAGTATATTT CAACGAGAAA
 301   GGAGTACTAA CAGCCAGAGA AATCACCCTC AAAGCCGGCG ACAACCTGAA
 351   AATCAAACAA AACGGCACAA ACTTCACCTA CTCGCTGAAA AAAGACCTCA
 401   CAGATCTGAC CAGTGTTGGA ACTGAAAAAT TATCGTTTAG CGCAAACGGC
 451   AATAAAGTCA ACATCACAAG CGACACCAAA GGCTTGAATT TTGCGAAAGA
 501   AACGGCTGGG ACGAACGGCG ACACCACGGT TCATCTGAAC GGTATTGGTT
 551   CGACTTTGAC CGATACGCTG CTGAATACCG GAGCGACCAC AAACGTAACC
 601   AACGACAACG TTACCGATGA CGAGAAAAAA CGTGCGGCAA GCGTTAAAGA
 651   CGTATTAAAC GCTGGCTGGA ACATTAAAGG CGTTAAACCC GGTACAACAG
 701   CTTCCGATAA CGTTGATTTC GTCCGCACTT ACGACACAGT CGAGTTCTTG
 751   AGCGCAGATA CGAAAACAAC GACTGTTAAT GTGGAAAGCA AAGACAACGG
 801   CAAGAAAACC GAAGTTAAAA TCGGTGCGAA GACTTCTGTT ATTAAAGAAA
 851   AAGACGGTAA GTTGGTTACT GGTAAAGACA AAGGCGAGAA TGGTTCTTCT
 901   ACAGACGAAG GCGAAGGCTT AGTGACTGCA AAAGAAGTGA TTGATGCAGT
 951   AAACAAGGCT GGTTGGAGAA TGAAAACAAC AACCGCTAAT GGTCAAACAG
1001   GTCAAGCTGA CAAGTTTGAA ACCGTTACAT CAGGCACAAA TGTAACCTTT
1051   GCTAGTGGTA AAGGTACAAC TGCGACTGTA AGTAAAGATG ATCAAGGCAA
1101   CATCACTGTT ATGTATGATG TAAATGTCGG CGATGCCCTA AACGTCAATC
1151   AGCTGCAAAA CAGCGGTTGG AATTTGGATT CCAAAGCGGT TGCAGGTTCT
1201   TCGGGCAAAG TCATCAGCGG CAATGTTTCG CCGAGCAAGG GAAAGATGGA
1251   TGAAACCGTC AACATTAATG CCGGCAACAA CATCGAGATT ACCCGCAACG
1301   GTAAAAATAT CGACATCGCC ACTTCGATGA CCCCGCAGTT TTCCAGCGTT
1351   TCGCTCGGCG CGGGGGCGGA TGCGCCCACT TTGAGCGTGG ATGGGGACGC
1401   ATTGAATGTC GGCAGCAAGA AGGACAACAA ACCCGTCCGC ATTACCAATG
1451   TCGCCCCGGG CGTTAAAGAG GGGGATGTTA CAAACGTCGC ACAACTTAAA
1501   GGCGTGGCGC AAAACTTGAA CAACCGCATC GACAATGTGG ACGGCAACGC
1551   GCGTGCGGGC ATCGCCCAAG CGATTGCAAC CGCAGGTCTG GTTCAGGCGT
1601   ATTTGCCCGG CAAGAGTATG ATGGCGATCG GCGGCGGCAC TTATCGCGGC
1651   GAAGCCGGTT ACGCCATCGG CTACTCCAGT ATTTCCGACG GCGGAAATTG
1701   GATTATCAAA GGCACGGCTT CCGGCAATTC GCGCGGCCAT TTCGGTGCTT
1751   CCGCATCTGT CGGTTATCAG TGGTAA
```

This corresponds to the amino acid sequence <SEQ ID 4; ORF40-1>:

```
  1   MNKIYRIIWN SALNAWVVVS ELTRNHTKRA SATVKTAVLA TLLFATVQAS
 51   ANNEEQEEDL YLDPVQRTVA VLIVNSDKEG TGEKEKVEEN SDWAVYFNEK
101   GVLTAREITL KAGDNLKIKQ NGTNFTYSLK KDLTDLTSVG TEKLSFSANG
151   NKVNITSDTK GLNFAKETAG TNGDTTVHLN GIGSTLTDTL LNTGATTNVT
201   NDNVTDDEKK RAASVKDVLN AGWNIKGVKP GTTASDNVDF VRTYDTVEFL
251   SADTKTTTVN VESKDNGKKT EVKIGAKTSV IKEKDGKLVT GKDKGENGSS
301   TDEGEGLVTA KEVIDAVNKA GWRMKTTTAN GQTGQADKFE TVTSGTNVTF
351   ASGKGTTATV SKDDQGNITV MYDVNVGDAL NVNQLQNSGW NLDSKAVAGS
401   SGKVISGNVS PSKGKMDETV NINAGNNIEI TRNGKNIDIA TSMTPQFSSV
451   SLGAGADAPT LSVDGDALNV GSKKDNKPVR ITNVAPGVKE GDVTNVAQLK
501   GVAQNLNNRI DNVDGNARAG IAQAIATAGL VQAYLPGKSM MAIGGGTYRG
551   EAGYAIGYSS ISDGGNWIIK GTASGNSRGH FGASASVGYQ W*
```

Further work identified the corresponding gene in strain A of *N.meningitidis* <SEQ ID 5>:

```
  1   ATGAACAAAA TATACCGCAT CATTTGGAAT AGTGCCCTCA ATGCCTGNGT
 51   CGCCGTATCC GAGCTCACAC GCAACCACAC CAAACGCGCC TCCGCAACCG
101   TGAAGACCGC CGTATTGGCG ACACTGTTGT TTGCAACGGT TCAGGCGAAT
151   GCTACCGATG AAGATGAAGA AGAAGAGTTA GAATCCGTAC AACGCTCTGT
201   CGTAGGGAGC ATTCAAGCCA GTATGGAAGG CAGCGGCGAA TTGGAAACGA
251   TATCATTATC AATGACTAAC GACAGCAAGG AATTTGTAGA CCCATACATA
301   GTAGTTACCC TCAAAGCCGG CGACAACCTG AAAATCAAAC AAAACACCAA
351   TGAAAACACC AATGCCAGTA GCTTCACCTA CTCGCTGAAA AAAGACCTCA
401   CAGGCCTGAT CAATGTTGAN ACTGAAAAAT TATCGTTTGG CGCAAACGGC
451   AAGAAAGTCA ACATCATAAG CGACACCAAA GGCTTGAATT TCGCGAAAGA
501   AACGGCTGGG ACGAACGGCG ACACCACGGT TCATCTGAAC GGTATCGGTT
551   CGACTTTGAC CGATACGCTT GCGGGTTCTT CTGCTTCTCA CGTTGATGCG
601   GGTAACCNAA GTACACATTA CACTCGTGCA GCAAGTATTA AGGATGTGTT
```

```
 651  GAATGCGGGT TGGAATATTA AGGGTGTTAA ANNNGGCTCA ACAACTGGTC
 701  AATCAGAAAA TGTCGATTTC GTCCGCACTT ACGACACAGT CGAGTTCTTG
 751  AGCGCAGATA CGNAAACAAC GACNGTTAAT GTGGAAAGCA AAGACAACGG
 801  CAAGAGAACC GAAGTTAAAA TCGGTGCGAA GACTTCTGTT ATTAAAGAAA
 851  AAGACGGTAA GTTGGTTACT GGTAAAGGCA AAGGCGAGAA TGGTTCTTCT
 901  ACAGACGAAG GCGAAGGCTT AGTGACTGCA AAAGAAGTGA TTGATGCAGT
 951  AAACAAGGCT GGTTGGAGAA TGAAAACAAC AACCGCTAAT GGTCAAACAG
1001  GTCAAGCTGA CAAGTTTGAA ACCGTTACAT CAGGCACAAA TGTAACCTTT
1051  GCTAGTGGTA AAGGTACAAC TGCGACTGTA AGTAAAGATG ATCAAGGCAA
1101  CATCACTGTT ATGTATGATG TAAATGTCGG CGATGCCCTA AACGTCAATC
1151  AGCTGCAAAA CAGCGGTTGG AATTTGGATT CCAAAGCGGT TGCAGGTTCT
1201  TCGGGCAAAG TCATCAGCGG CAATGTTTCG CCGAGCAAGG GAAAGATGGA
1251  TGAAACCGTC AACATTAATG CCGGCAACAA CATCGAGATT AGCCGCAACG
1301  GTAAAAATAT CGACATCGCC ACTTCGATGG CGCCGCAGTT TTCCAGCGTT
1351  TCGCTCGGCG CGGGGGCAGA TGCGCCCACT TTAAGCGTGG ATGACGAGGG
1401  CGCGTTGAAT GTCGGCAGCA AGGATGCCAA CAAACCCGTC CGCATTACCA
1451  ATGTCGCCCC GGGCGTTAAA GANGGGGATG TTACAAACGT CNCACAACTT
1501  AAAGGCGTGG CGCAAAACTT GAACAACCGC ATCGACAATG TGGACGGCAA
1551  CGCGCGTGCN GGCATCGCCC AAGCGATTGC AACCGCAGGT CTGGTTCAGG
1601  CGTATCTGCC CGGCAAGAGT ATGATGGCGA TCGGCGGCGG CACTTATCGC
1651  GGCGAAGCCG GTTACGCCAT CGGCTACTCC AGTATTTCCG ACGGCGGAAA
1701  TTGGATTATC AAAGGCACGG CTTCCGGCAA TTCGCGCGGC CATTTCGGTG
1751  CTTCCGCATC TGTCGGTTAT CAGTGGTAA
```

This encodes a protein having amino acid sequence <SEQ ID 6; ORF40a>:

```
  1  MNKIYRIIWN SALNAXVAVS ELTRNHTKRA SATVKTAVLA TLLFATVQAN
 51  ATDEDEEEEL ESVQRSVVGS IQASMEGSGE LETISLSMTN DSKEFVDPYI
101  VVTLKAGDNL KIKQNTNENT NASSFTYSLK KDLTGLINVX TEKLSFGANG
151  KKVNIISDTK GLNFAKETAG TNGDTTVHLN GIGSTLTDTL AGSSASHVDA
201  GNXSTHYTRA ASIKDVLNAG WNIKGVKXGS TTGQSENVDF VRTYDTVEFL
251  SADTXTTTVN VESKDNGKRT EVKIGAKTSV IKEKDGKLVT GKGKGENGSS
301  TDEGEGLVTA KEVIDAVNKA GWRMKTTTAN GQTGQADKFE TVTSGTNVTF
351  ASGKGTTATV SKDDQGNITV MYDVNVGDAL NVNQLQNSGW NLDSKAVAGS
401  SGKVISGNVS PSKGKMDETV NINAGNNIEI SRNGKNIDIA TSMAPQFSSV
451  SLGAGADAPT LSVDDEGALN VGSKDANKPV RITNVAPGVK XGDVTNVXQL
501  KGVAQNLNNR IDNVDGNARA GIAQAIATAG LVQAYLPGKS MMAIGGGTYR
551  GEAGYAIGYS SISDGGNWII KGTASGNSRG HFGASASVGY QW*
```

The originally-identified partial strain B sequence (ORF40) shows 65.7% identity over a 254aa overlap with ORF40a:

```
                                                   10        20        30
orf40.pep                                  TLLFATVQASANQEEQEEDLYLDPVQRTVA
                                           |||||||||:|::|::|||:|   : ||||:|
orf40a       SALNAXVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL--ESVQRSV-
                     20        30        40        50        60

                     40        50        60        70        80
orf40.pep    VLIVNSDKEGTGEKEKVEEN-SDWAVYFNEKGVLTAREITXKAGDNLKIKQN------GT
             |  :::: ||:|| | :   : :: :   | :   ::     :| |||||||||||       ::
orf40a       VGSIQASMEGSGELETISLSMTNDSKEFVDPYIV----VTLKAGDNLKIKQNTNENTNAS
              70        80        90       100            110       120

                 90       100       110       120       130       140
orf40.pep    NFTYSLKKDLTDLTSVGTEKLSFSANGNKVNITSDTKGLNFAKETAGTNGDTTVHLNGIG
             :||||||||||  | :| ||||||:|||:|||| |||||||||||||||||||||||||||
orf40a       SFTYSLKKDLTGLINVXTEKLSFGANGKKVNIISDTKGLNFAKETAGTNGDTTVHLNGIG
                 130       140       150       160       170       180

                 150       160       170       180       190         200
orf40.pep    STLTDTLLNTGATTNVTNDNVTDDEKKRAASVKDVLNAGWNIKGVKPGTTA--SDNVDFV
```



```
             ||||||| :::|: :|    | :  :  ||||:|||||||||||||| |:|:  |:|||||
orf40a       STLTDTLAGSSAS-HVDAGNXST-HYTRAASIKDVLNAGWNIKGVKXGSTTGQSENVDFV
                 190       200       210       220       230       240

                 210       220       230       240
orf40.pep    RTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKD
             |||||||||||||| |||||||||||||:||||||||||||||||
orf40a       RTYDTVEFLSADTXTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTGKGKGENGSST
                 250       260       270       280       290       300
```

The complete strain B sequence (ORF40-1) and ORF40a show 83.7% identity in 601 aa overlap:

```
                     10        20        30        40        50        60
orf40-1.pep  MNKIYRIIWNSALNAWVVVSELTRNHTKRASATVKTAVLATLLFATVQASANNEEQEEDL
             |||||||||||||||| |:||||||||||||||||||||||||||||||||||||:|::|::|||:|
orf40a       MNKIYRIIWNSALNAXVAVSELTRNHTKRASATVKTAVLATLLFATVQANATDEDEEEEL
                     10        20        30        40        50        60

                     70        80        90       100       110      119
orf40-1.pep  YLDPVQRTVAVLIVNSDKEGTGEKEKVEEN-SDWAVYFNEKGVLTAREITLKAGDNLKIK
               : ||||:|| |  :::: ||:|| | :   : :: :   | :   ::     :|||||||||||
orf40a       --ESVQRSV-VGSIQASMEGSGELETISLSMTNDSKEFVDPYIV----VTLKAGDNLKIK
                       70        80        90       100            110

          120        130       140       150       160       170
orf40-1.pep  QN------GTNFTYSLKKDLTDLTSVGTEKLSFSANGNKVNITSDTKGLNFAKETAGTNG
             ||       ::||||||||||| | :| ||||||:|||:|||| ||||||||||||||||||
orf40a       QNTNENTNASSFTYSLKKDLTGLINVXTEKLSFGANGKKVNIISDTKGLNFAKETAGTNG
                  120       130       140       150       160       170

                 180       190       200       210       220       230
orf40-1.pep  DTTVHLNGIGSTLTDTLLNTGATTNVTNDNVTDDEKKRAASVKDVLNAGWNIKGVKPGTT
             |||||||||||||||||| :::|: :|    | :  :  ||||:|||||||||||||| |:|
orf40a       DTTVHLNGIGSTLTDTLAGSSAS-HVDAGNXST-HYTRAASIKDVLNAGWNIKGVKXGST
                 180       190       200       210       220       230

                 240       250       260       270       280       290
orf40-1.pep  A--SDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKIGAKTSVIKEKDGKLVTG
             :  |:|||||||||||||||||||| |||||||||||||:||||||||||||||||||||||||
orf40a       TGQSENVDFVRTYDTVEFLSADTXTTTVNVESKDNGKRTEVKIGAKTSVIKEKDGKLVTG
                 240       250       260       270       280       290

                 300       310       320       330       340       350
orf40-1.pep  KDKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFA
             | |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf40a       KGKGENGSSTDEGEGLVTAKEVIDAVNKAGWRMKTTTANGQTGQADKFETVTSGTNVTFA
                 300       310       320       330       340       350

                 360       370       380       390       400       410
orf40-1.pep  SGKGTTATVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSP
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf40a       SGKGTTATVSKDDQGNITVMYDVNVGDALNVNQLQNSGWNLDSKAVAGSSGKVISGNVSP
                 360       370       380       390       400       410

                 420       430       440       450       460       470
orf40-1.pep  SKGKMDETVNINAGNNIEITRNGKNIDIATSMTPQFSSVSLGAGADAPTLSVDGD-ALNV
             ||||||||||||||||||:||||||||||||:|||||||||||||||||||||| : ||||
orf40a       SKGKMDETVNINAGNNIEISRNGKNIDIATSMAPQFSSVSLGAGADAPTLSVDDEGALNV
                 420       430       440       450       460       470

                 480       490       500       510       520       530
orf40-1.pep  GSKKDNKPVRITNVAPGVKEGDVTNVAQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGL
             |||  ||||||||||||| |||||| ||||||||||||||||||||||||||||||||||
```

```
orf40a       GSKDANKPVRITNVAPGVKXGDVTNVXQLKGVAQNLNNRIDNVDGNARAGIAQAIATAGL
                     480       490       500       510       520       530

                     540       550       560       570       580       590
orf40-1.pep  VQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQ
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf40a       VQAYLPGKSMMAIGGGTYRGEAGYAIGYSSISDGGNWIIKGTASGNSRGHFGASASVGYQ
                     540       550       560       570       580       590

orf40-1.pep  WX
             ||
orf40a       WX
```

Computer analysis of these amino acid sequences gave the following results:

<u>Homology with Hsf protein encoded by the type b surface fibrils locus of *H.influenzae* (accession number U41852)</u>

ORF40 and Hsf protein show 54% aa identity in 251 aa overlap:

```
Orf40   1   TLLFATVQASANQEEQEEDLYLDPVQRTVAVLIVNSDXXXXXXXXXXXXNSDWAVYFNEK 60
            TLLFATVQA+A  E++E    LDPV RT  VL  +SD             NS+W +YF+ K
Hsf     41  TLLFATVQANATDEDEE----LDPVVRTAPVLSFHSDKEGTGEKEVTE-NSNWGIYFDNK 95

Orf40   61  GVLTAREITXKAGDNLKIKQN------GTNFTYSLKKDLTDLTSVGTEKLSFSANGNKVN 114
            GVL A  IT KAGDNLKIKQN       ++FTYSLKKDLTDLTSV TEKLSF ANG+KV+
Hsf     96  GVLKAGAITLKAGDNLKIKQNTDESTNASSFTYSLKKDLTDLTSVATEKLSFGANGDKVD 155

Orf40   115 ITSDTKGLNFAKETAGTNGDTTVHLNGIGSTLTDTLLNTGAXXXXXXXXXXXXEKKRAAS 174
            ITSD  GL  AK      G+  VHLNG+ STL D + NTG              EK RAA+
Hsf     156 ITSDANGLKLAK-----TGNGNVHLNGLDSTLPDAVTNTGVLSSSSFTPNDV-EKTRAAT 209

Orf40   175 VKDVLNAGWNIKGVKPGTTASDNVDFVRTYDTVEFLSADTKTTTVNVESKDNGKKTEVKI 234
            VKDVLNAGWNIKG K       ++VD V  Y+ VEF++ D  T  V + +K+NGK TEVK
Hsf     210 VKDVLNAGWNIKGAKTAGGNVESVDLVSAYNNVEFITGDKNTLDVVLTAKENGKTTEVKF 269

Orf40   235 GAKTSVIKEKD 245
              KTSVIKEKD
Hsf     270 TPKTSVIKEKD 280
```

ORF40a also shows homology to Hsf:

```
gi|1666683 (U41852) hsf gene product [Haemophilus influenzae] Length = 2353
 Score = 153 (67.7 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116
 Identities = 33/36 (91%), Positives = 34/36 (94%)

Query:    16 VAVSELTRNHTKRASATVKTAVLATLLFATVQANAT 51
             V VSELTR HTKRASATV+TAVLATLLFATVQANAT
Sbjct:    17 VVVSELTRTHTKRASATVETAVLATLLFATVQANAT 52

 Score = 161 (71.2 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116
 Identities = 32/38 (84%), Positives = 36/38 (94%)

Query:   101 VTLKAGDNLKIKQNTNENTNASSFTYSLKKDLTGDINV 138
             +TLKAGDNLKIKQNT+E+TNASSFTYSLKKDLT D +V
Sbjct:   103 ITLKAGDNLKIKQNTDESTNASSFTYSLKKDLTDDTSV 140

 Score = 110 (48.7 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116
 Identities = 21/29 (72%), Positives = 25/29 (86%)

Query:   138 VTEKLSFGANGKKVNIISDTKGLNFAKET 166
             V++KLS G NG KVNI SDTKGLNFAK++
Sbjct:  1439 VSDKLSLGTNGNKVNITSDTKGLNFAKDS 1467
```



```
 Score = 85 (37.6 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116
 Identities = 18/32 (56%), Positives = 20/32 (62%)

Query:   169 TNGDTTVHLNGIGSTLTDTLAGSSASHVDAGN 200
             T  D  +HLNGI STLTDTL  S A+    GN
Sbjct:  1469 TGDDANIHLNGIASTLTDTLLNSGATTNLGGN 1500

 Score = 92 (40.7 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116
 Identities = 16/19 (84%), Positives = 19/19 (100%)

Query:   206 RAASIKDVLNAGWNIKGVK 224
             RAAS+KDVLNAGWN++GVK
Sbjct:  1509 RAASVKDVLNAGWNVRGVK 1527

 Score = 90 (39.8 bits), Expect = 1.5e-116, Sum P(11) = 1.5e-116
 Identities = 17/28 (60%), Positives = 20/28 (71%)

Query:   226 STTGQSENVDFVRTYDTVEFLSADTTTT 253
             S   Q EN+DFV TYDTV+F+S D  TT
Sbjct:  1530 SANNQVENIDFVATYDTVDFVSGDKDTT 1557
```

Based on homology with Hsf, it was predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

ORF40-1 (61kDa) was cloned in pET and pGex vectors and expressed in *E.coli*, as described above. The products of protein expression and purification were analyzed by SDS-PAGE. Figure 1A shows the results of affinity purification of the His-fusion protein, and Figure 1B shows the results of expression of the GST-fusion in *E.coli*. Purified His-fusion protein was used to immunise mice, whose sera were used for FACS analysis (Figure 1C), a bactericidal assay (Figure 1D), and ELISA (positive result). These experiments confirm that ORF40-1 is a surface-exposed protein, and that it is a useful immunogen.

Figure 1E shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF40-1.

Example 2

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 7>

```
  1  ATGTTACGTt TGACTGCtTT AGCCGTATGC ACCGCCCTCG CTTTGGGCGC
 51  GTGTTCGCCG CAAAATTCCG ACTCTGCCCC ACAAGCCAAA GaACAGGCGG
101  TTTCCGCCGC ACAAACCGAA GgCGCGTCCG TTACCGTCAA AACCGCGCGC
151  GGCGACGTTC AAATACCGCA AAACCCCGAA CGCATCGCCG TTTACGATTT
201  GGGTATGCTC GACACCTTGA GCAAACTGGG CGTGAAAACC GGTTTGTCCG
251  TCGATAAAAA CCGCCTGCCG TATTTAGAGG AATATTTCAA AACGACAAAA
301  CCTGCCGGCA CTTTGTTCGA GCCGGATTAC GAAACGCTCA ACGCTTACAA
351  ACCGCAGCTC ATCATCATCG GCAGCCGCGC CgCCAAGGCG TTTGACAAAT
401  TGAAcGAAAT CGCGCCGACC ATCGrmwTGA CCGCCGATAC CGCCAACCTC
451  AAAGAAAGTG CCAArGAGGC ATCGACGCTG GCGCAAATCT TC..
```

This corresponds to the amino acid sequence <SEQ ID 8; ORF38>:

```
  1  MLRLTALAVC TALALGACSP QNSDSAPQAK EQAVSAAQTE GASVTVKTAR
 51  GDVQIPQNPE RIAVYDLGML DTLSKLGVKT GLSVDKNRLP YLEEYFKTTK
101  PAGTLFEPDY ETLNAYKPQL IIIGSRAAKA FDKLNEIAPT IXXTADTANL
151  KESAKEASTL AQIF..
```

Further work revealed the complete nucleotide sequence <SEQ ID 9>:

```
  1  ATGTTACGTT TGACTGCTTT AGCCGTATGC ACCGCCCTCG CTTTGGGCGC
 51  GTGTTCGCCG CAAAATTCCG ACTCTGCCCC ACAAGCCAAA GAACAGGCGG
101  TTTCCGCCGC ACAAACCGAA GGCGCGTCCG TTACCGTCAA AACCGCGCGC
151  GGCGACGTTC AAATACCGCA AAACCCCGAA CGCATCGCCG TTTACGATTT
201  GGGTATGCTC GACACCTTGA GCAAACTGGG CGTGAAAACC GGTTTGTCCG
251  TCGATAAAAA CCGCCTGCCG TATTTAGAGG AATATTTCAA AACGACAAAA
301  CCTGCCGGCA CTTTGTTCGA GCCGGATTAC GAAACGCTCA ACGCTTACAA
351  ACCGCAGCTC ATCATCATCG GCAGCCGCGC CGCCAAGGCG TTTGACAAAT
401  TGAACGAAAT CGCGCCGACC ATCGAAATGA CCGCCGATAC CGCCAACCTC
451  AAAGAAAGTG CCAAAGAGCG CATCGACGCG CTGGCGCAAA TCTTCGGCAA
501  ACAGGCGGAA GCCGACAAGC TGAAGGCGGA AATCGACGCG TCTTTTGAAG
551  CCGCGAAAAC TGCCGCACAA GGTAAGGGCA AAGGTTTGGT GATTTTGGTC
601  AACGGCGGCA AGATGTCGGC TTTCGGCCCG TCTTCACGCT TGGGCGGCTG
651  GCTGCACAAA GACATCGGCG TTCCCGCTGT CGATGAATCA ATTAAAGAAG
701  GCAGCCACGG TCAGCCTATC AGCTTTGAAT ACCTGAAAGA GAAAAATCCC
751  GACTGGCTGT TTGTCCTTGA CCGAAGCGCG GCCATCGGCG AAGAGGGTCA
801  GGCGGCGAAA GACGTGTTGG ATAATCCGCT GGTTGCCGAA ACAACCGCTT
851  GGAAAAAAGG ACAGGTCGTG TACCTCGTTC CTGAAACTTA TTTGGCAGCC
901  GGTGGCGCGC AAGAGCTGCT GAATGCAAGC AAACAGGTTG CCGACGCTTT
951  TAACGCGGCA AAATAA
```

This corresponds to the amino acid sequence <SEQ ID 10; ORF38-1>:

```
  1  MLRLTALAVC TALALGACSP QNSDSAPQAK EQAVSAAQTE GASVTVKTAR
 51  GDVQIPQNPE RIAVYDLGML DTLSKLGVKT GLSVDKNRLP YLEEYFKTTK
101  PAGTLFEPDY ETLNAYKPQL IIIGSRAAKA FDKLNEIAPT IEMTADTANL
151  KESAKERIDA LAQIFGKQAE ADKLKAEIDA SFEAAKTAAQ GKGKGLVILV
201  NGGKMSAFGP SSRLGGWLHK DIGVPAVDES IKEGSHGQPI SFEYLKEKNP
251  DWLFVLDRSA AIGEEGQAAK DVLDNPLVAE TTAWKKGQVV YLVPETYLAA
301  GGAQELLNAS KQVADAFNAA K*
```

Computer analysis of this amino acid sequence reveals a putative prokaryotic membrane lipoprotein lipid attachment site (underlined).

Further work identified the corresponding gene in strain A of *N.meningitidis* <SEQ ID 11>:

```
  1  ATGTTACGTT TGACTGCTTT AGCCGTATGC ACCGCCCTCG CTTTGGGCGC
 51  GTGTTCGCCG CAAAATTCCG ACTCTGCCCC ACAAGCCAAA GAACAGGCGG
101  TTTCCGCCGC ACAATCCGAA GGCGTGTCCG TTACCGTCAA AACGGCGCGC
151  GGCGATGTTC AAATACCGCA AAACCCCGAA CGTATCGCCG TTTACGATTT
201  GGGTATGCTC GACACCTTGA GCAAACTGGG CGTGAAAACC GGTTTGTCCG
251  TCGATAAAAA CCGCCTGCCG TATTTAGAGG AATATTTCAA AACGACAAAA
301  CCTGCCGGAA CTTTGTTCGA GCCGGATTAC GAAACGCTCA ACGCTTACAA
351  ACCGCAGCTC ATCATCATCG GCAGCCGCGC AGCCAAAGCG TTTGACAAAT
401  TGAACGAAAT CGCGCCGACC ATCGAAATGA CCGCCGATAC CGCCAACCTC
451  AAAGAAAGTG CCAAAGAGCG TATCGACGCG CTGGCGCAAA TCTTCGGCAA
501  AAAGGCGGAA GCCGACAAGC TGAAGGCGGA AATCGACGCG TCTTTTGAAG
551  CCGCGAAAAC TGCCGCGCAA GGCAAAGGCA AGGGTTTGGT GATTTTGGTC
601  AACGGCGGCA AGATGTCCGC CTTCGGCCCG TCTTCACGAC TGGGCGGCTG
651  GCTGCACAAA GACATCGGCG TTCCCGCTGT TGACGAAGCC ATCAAAGAAG
701  GCAGCCACGG TCAGCCTATC AGCTTTGAAT ACCTGAAAGA GAAAAATCCC
751  GACTGGCTGT TTGTCCTTGA CCGCAGCGCG GCCATCGGCG AAGAGGGTCA
801  GGCGGCGAAA GACGTGTTGA ACAATCCGCT GGTTGCCGAA ACAACCGCTT
```



```
851  GGAAAAAAGG ACAAGTCGTT TACCTTGTTC CTGAAACTTA TTTGGCAGCC
901  GGTGGCGCGC AAGAGCTACT GAATGCAAGC AAACAGGTTG CCGACGCTTT
951  TAACGCGGCA AAATAA
```

This encodes a protein having amino acid sequence <SEQ ID 12; ORF38a>:

```
  1  MLRLTALAVC TALALGACSP QNSDSAPQAK EQAVSAAQSE GVSVTVKTAR
 51  GDVQIPQNPE RIAVYDLGML DTLSKLGVKT GLSVDKNRLP YLEEYFKTTK
101  PAGTLFEPDY ETLNAYKPQL IIIGSRAAKA FDKLNEIAPT IEMTADTANL
151  KESAKERIDA LAQIFGKKAE ADKLKAEIDA SFEAAKTAAQ GKGKGLVILV
201  NGGKMSAFGP SSRLGGWLHK DIGVPAVDEA IKEGSHGQPI SFEYLKEKNP
251  DWLFVLDRSA AIGEEGQAAK DVLNNPLVAE TTAWKKGQVV YLVPETYLAA
301  GGAQELLNAS KQVADAFNAA K*
```

The originally-identified partial strain B sequence (ORF38) shows 95.2% identity over a 165aa overlap with ORF38a:

```
                      10        20        30        40        50        60
orf38.pep     MLRLTALAVCTALALGACSPQNSDSAPQAKEQAVSAAQTEGASVTVKTARGDVQIPQNPE
              ||||||||||||||||||||||||||||||||||||||||||:|||:|||||||||||||
orf38a        MLRLTALAVCTALALGACSPQNSDSAPQAKEQAVSAAQSEGVSVTVKTARGDVQIPQNPE
                      10        20        30        40        50        60

                      70        80        90       100       110       120
orf38.pep     RIAVYDLGMLDTLSKLGVKTGLSVDKNRLPYLEEYFKTTKPAGTLFEPDYETLNAYKPQL
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf38a        RIAVYDLGMLDTLSKLGVKTGLSVDKNRLPYLEEYFKTTKPAGTLFEPDYETLNAYKPQL
                      70        80        90       100       110       120

                     130       140       150       160
orf38.pep     IIIGSRAAKAFDKLNEIAPTIXXTADTANLKESAKE-ASTLAQIF
              |||||||||||||||||||||  |||||||||||||   ::|||||
orf38a        IIIGSRAAKAFDKLNEIAPTIEMTADTANLKESAKERIDALAQIFGKKAEADKLKAEIDA
                     130       140       150       160       170       180

orf38a        SFEAAKTAAQGKGKGLVILVNGGKMSAFGPSSRLGGWLHKDIGVPAVDEAIKEGSHGQPI
                     190       200       210       220       230       240
```

The complete strain B sequence (ORF38-1) and ORF38a show 98.4% identity in 321 aa overlap:

```
orf38a.pep    MLRLTALAVCTALALGACSPQNSDSAPQAKEQAVSAAQSEGVSVTVKTARGDVQIPQNPE
              ||||||||||||||||||||||||||||||||||||||:|||:|||||||||||||||||
orf38-1       MLRLTALAVCTALALGACSPQNSDSAPQAKEQAVSAAQTEGASVTVKTARGDVQIPQNPE

orf38a.pep    RIAVYDLGMLDTLSKLGVKTGLSVDKNRLPYLEEYFKTTKPAGTLFEPDYETLNAYKPQL
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf38-1       RIAVYDLGMLDTLSKLGVKTGLSVDKNRLPYLEEYFKTTKPAGTLFEPDYETLNAYKPQL

orf38a.pep    IIIGSRAAKAFDKLNEIAPTIEMTADTANLKESAKERIDALAQIFGKKAEADKLKAEIDA
              ||||||||||||||||||||||||||||||||||||||||||||||:|||||||||||||
orf38-1       IIIGSRAAKAFDKLNEIAPTIEMTADTANLKESAKERIDALAQIFGKQAEADKLKAEIDA

orf38a.pep    SFEAAKTAAQGKGKGLVILVNGGKMSAFGPSSRLGGWLHKDIGVPAVDEAIKEGSHGQPI
              ||||||||||||||||||||||||||||||||||||||||||||||||||:|||||||||
orf38-1       SFEAAKTAAQGKGKGLVILVNGGKMSAFGPSSRLGGWLHKDIGVPAVDESIKEGSHGQPI

orf38a.pep    SFEYLKEKNPDWLFVLDRSAAIGEEGQAAKDVLNNPLVAETTAWKKGQVVYLVPETYLAA
              |||||||||||||||||||||||||||||||||:||||||||||||||||||||||||||
orf38-1       SFEYLKEKNPDWLFVLDRSAAIGEEGQAAKDVLDNPLVAETTAWKKGQVVYLVPETYLAA

orf38a.pep    GGAQELLNASKQVADAFNAAK
              |||||||||||||||||||||
```

```
orf38-1        GGAQELLNASKQVADAFNAAK
```

Computer analysis of these sequences revealed the following:

Homology with a lipoprotein (lipo) of *C.jejuni* (accession number X82427)

ORF38 and lipo show 38% aa identity in 96 aa overlap:

```
Orf38: 40  EGASVTVKTARGDVQIPQNPERIAVYDLGMLDTLSKLGVKTGLS-VDKNRLPYLEEYFKT 98
           EG S  VK + G+ + P+NP ++ + DLG+LDT   L +    ++ V    LP   + FK
Lipo:  51  EGDSFLVKDSLGENKTPKNPSKVVILDLGILDTFDALKLNDKVAGVPAKNLPKYLQQFKN 110

Orf38: 99  TKPAGTLFEPDYETLNAYKPQLIIIGSRAAKAFDKL 134
               G + + D+E +NA KP LIII  R +K +DKL
Lipo:  111 KPSVGGVQQVDFEAINALKPDLIIISGRQSKFYDKL 146
```

Based on this analysis, it was predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

ORF38-1 (32kDa) was cloned in pET and pGex vectors and expressed in *E.coli*, as described above. The products of protein expression and purification were analyzed by SDS-PAGE. Figure 2A shows the results of affinity purification of the His-fusion protein, and Figure 2B shows the results of expression of the GST-fusion in *E.coli*. Purified His-fusion protein was used to immunise mice, whose sera were used for Western blot analysis (Figure 2C) and FACS analysis (Figure 2D). These experiments confirm that ORF38-1 is a surface-exposed protein, and that it is a useful immunogen.

Figure 2E shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF38-1.

Example 3

The following *N.meningitidis* DNA sequence was identified <SEQ ID 13>:

```
  1 ATGAAACTTC TGACCACCGC AATCCTGTCT TCCGCAATCG CGCTCAGCAG
 51 TATGGCTGCC GCCGCTGGCA CGGACAACCC CACTGTTGCA AAAAAAACCG
101 TCAGCTACGT CTGCCAGCAA GGTAAAAAAG TCAAAGTAAC CTACGGCTTC
151 AACAAACAGG GTCTGACCAC ATACGCTTCC GCCGTCATCA ACGGCAAACG
201 CGTGCAAATG CCTGTCAATT TGGACAAATC CGACAATGTG GAAACATTCT
251 ACGGCAAAGA AGGCGGTTAT GTTTTGGGTA CCGGCGTGAT GGATGGCAAA
301 TCCTACCGCA AACAGCCCAT TATGATTACC GCACCTGACA ACCAAATCGT
351 CTTCAAAGAC TGTTCCCCAC GTTAA
```

This corresponds to the amino acid sequence <SEQ ID 14; ORF44>:

```
  1 MKLLTTAILS SAIALSSMAA AAGTDNPTVA KKTVSYVCQQ GKKVKVTYGF
 51 NKQGLTTYAS AVINGKRVQM PVNLDKSDNV ETFYGKEGGY VLGTGVMDGK
101 SYRKQPIMIT APDNQIVFKD CSPR*
```

Computer analysis of this amino acid sequence predicted the leader peptide shown underlined.

Further work identified the corresponding gene in strain A of *N.meningitidis* <SEQ ID 15>:

```
  1 ATGAAACTTC TGACCACCGC AATCCTGTCT TCCGCAATCG CGCTCAGCAG
 51 TATGGCTGCT GCTGCCGGCA CGAACAACCC CACCGTTGCC AAAAAAACCG
101 TCAGCTACGT CTGCCAGCAA GGTAAAAAAG TCAAAGTAAC CTACGGCTTT
151 AACAAACAGG GCCTGACCAC ATACGCTTCC GCCGTCATCA ACGGCAAACG
201 TGTGCAAATG CCTGTCAATT TGGACAAATC CGACAATGTG GAAACATTCT
251 ACGGCAAAGA AGGCGGTTAT GTTTTGGGTA CCGGCGTGAT GGATGGCAAA
301 TCCTATCGCA AACAGCCTAT TATGATTACC GCACCTGACA ACCAAATCGT
351 CTTCAAAGAC TGTTCCCCAC GTTAA
```

This encodes a protein having amino acid sequence <SEQ ID 16; ORF44a>:

```
  1 MKLLTTAILS SAIALSSMAA AAGTNNPTVA KKTVSYVCQQ GKKVKVTYGF
 51 NKQGLTTYAS AVINGKRVQM PVNLDKSDNV ETFYGKEGGY VLGTGVMDGK
101 SYRKQPIMIT APDNQIVFKD CSPR*
```

The strain B sequence (ORF44) shows 99.2% identity over a 124aa overlap with ORF44a:

```
                    10        20        30        40        50        60
orf44.pep   MKLLTTAILSSAIALSSMAAAAGTDNPTVAKKTVSYVCQQGKKVKVTYGFNKQGLTTYAS
            ||||||||||||||||||||||||:|||||||||||||||||||||||||||||||||||
orf44a      MKLLTTAILSSAIALSSMAAAAGTNNPTVAKKTVSYVCQQGKKVKVTYGFNKQGLTTYAS
                    10        20        30        40        50        60

                    70        80        90       100       110       120
orf44.pep   AVINGKRVQMPVNLDKSDNVETFYGKEGGYVLGTGVMDGKSYRKQPIMITAPDNQIVFKD
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf44a      AVINGKRVQMPVNLDKSDNVETFYGKEGGYVLGTGVMDGKSYRKQPIMITAPDNQIVFKD
                    70        80        90       100       110       120

orf44.pep   CSPRX
            |||||
orf44a      CSPRX
```

Computer analysis gave the following results:

Homology with the LecA adhesin of *Eikenella corrodens* (accession number D78153)

ORF44 and LecA protein show 45% aa identity in 91 aa overlap:

```
Orf44   33  TVSYVCQQGKKVKVTYGFNKQGLTTYASAVINGKRVQMPVNLDKSDNVETFYGKEGGYVL 92
            +V+YVCQQG+++ V Y FN  G+ T A   +N + +++P NL  SDNV+T +     GY L
LecA    135 SVAYVCQQGRRLNVNYRFNSAGVPTSAELRVNNRNLRLPYNLSASDNVDTVF-SANGYRL 193

Orf44   93  GTGVMDGKSYRKQPIMITAPDNQIVFKDCSP 123
             T  MD  +YR Q I+++AP+ Q+++KDCSP
LecA    194 TTNAMDSANYRSQDIIVSAPNGQMLYKDCSP 224
```

Based on homology with the adhesin, it was predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

ORF44-1 (11.2kDa) was cloned in pET and pGex vectors and expressed in *E.coli*, as described above. The products of protein expression and purification were analyzed by SDS-PAGE. Figure 3A shows the results of affinity purification of the His-fusion protein, and Figure 3B shows the results of expression of the GST-fusion in *E.coli*. Purified His-fusion protein was used to

immunise mice, whose sera were used for ELISA, which gave positive results, and for a bactericidal assay (Figure 3C). These experiments confirm that ORF44-1 is a surface-exposed protein, and that it is a useful immunogen.

Figure 3D shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF44-1.

Example 4

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 17>

```
  1  ..GGCACCGAAT TCAAAACCAC CCTTTCCGGA GCCGACATAC AGGCAGGGGT
 51    GGGTGAAAAA GCCCGAGCCG ATGCGAAAAT TATCCTAAAA GGCATCGTTA
101    ACCGCATCCA AACCGAAGAA AAGCTGGAAT CCAACTCGAC CGTATGGCAA
151    AAGCAGGCCG GAAGCGGCAG CACGGTTGAA ACGCTGAAGC TACCGAGCTT
201    TGAAGGGCCG GCACTGCCTA AGCTGACCGC TCCCGGCGGC TATATCGCCG
251    ACATCCCCAA AGGCAACCTC AAAACCGAAA TCGAAAAGCT GGCCAAACAG
301    CCCGAATATG CCTATCTGAA ACAGCTTCAG ACGGTCAAGG ACGTGAACTG
351    GAACCAAGTA CAGCTCGCTT ACGACAAATG GGACTATAAA CAGGAAGGCC
401    TAACCGGAGC CGGAGCCGCA ATTANCGCAC TGGCCGTTAC CGTGGTCACC
451    TCAGGCGCAG GAACCGGAGC CGTATTGGGA TTAANACGNG TGGCCGCCGC
501    CGCAACCGAT GCAGCATTT...
```

This corresponds to the amino acid sequence <SEQ ID 18; ORF49>:

```
  1  ..GTEFKTTLSG ADIQAGVGEK ARADAKIILK GIVNRIQTEE KLESNSTVWQ
 51    KQAGSGSTVE TLKLPSFEGP ALPKLTAPGG YIADIPKGNL KTEIEKLAKQ
101  . PEYAYLKQLQ TVKDVNWNQV QLAYDKWDYK QEGLTGAGAA IXALAVTVVT
151    SGAGTGAVLG LXRVAAAATD AAF..
```

Further work revealed the complete nucleotide sequence <SEQ ID 19>:

```
   1  ATGCAACTGC TGGCAGCCGA AGGCATTCAC CAACACCAAT TGAATGTTCA
  51  GAAAAGTACC CGTTTCATCG GCATCAAAGT GGGTAAAAGC AATTACAGCA
 101  AAAACGAGCT GAACGAAACC AAACTGCCCG TACGCGTTAT CGCCCAAACA
 151  GCCAAAACCC GTTCCGGCTG GGATACCGTA CTCGAAGGCA CCGAATTCAA
 201  AACCACCCTT TCCGGAGCCG ACATACAGGC AGGGGTGGGT GAAAAAGCCC
 251  GAGCCGATGC GAAAATTATC CTAAAAGGCA TCGTTAACCG CATCCAAACC
 301  GAAGAAAAGC TGGAATCCAA CTCGACCGTA TGGCAAAAGC AGGCCGGAAG
 351  CGGCAGCACG GTTGAAACGC TGAAGCTACC GAGCTTTGAA GGGCCGGCAC
 401  TGCCTAAGCT GACCGCTCCC GGCGGCTATA TCGCCGACAT CCCCAAAGGC
 451  AACCTCAAAA CCGAAATCGA AAAGCTGGCC AAACAGCCCG AATATGCCTA
 501  TCTGAAACAG CTTCAGACGG TCAAGGACGT GAACTGGAAC CAAGTACAGC
 551  TCGCTTACGA CAAATGGGAC TATAAACAGG AAGGCCTAAC CGGAGCCGGA
 601  GCCGCAATTA TCGCACTGGC CGTTACCGTG GTCACCTCAG GCGCAGGAAC
 651  CGGAGCCGTA TTGGGATTAA ACGGTGCGGC CGCCGCCGCA ACCGATGCAG
 701  CATTTGCCTC TTTGGCCAGC CAGGCTTCCG TATCGTTCAT CAACAACAAA
 751  GGCAATATCG GTAACACCCT GAAAGAGCTG GGCAGAAGCA GCACGGTGAA
 801  AAATCTGATG GTTGCCGTCG CTACCGCAGG CGTAGCCGAC AAAATCGGTG
 851  CTTCGGCACT GAACAATGTC AGCGATAAGC AGTGGATCAA CAACCTGACC
 901  GTCAACCTGG CCAATGCGGG CAGTGCCGCA CTGATTAATA CCGCTGTCAA
 951  CGGCGGCAGC CTGAAAGACA ATCTGGAAGC GAATATCCTT GCGGCTTTGG
1001  TGAATACTGC GCATGGAGAG GCAGCAAGTA AAATCAAACA GTTGGATCAG
1051  CACTACATTG CCCATAAGAT TGCCCATGCC ATAGCGGGCT GTGCGGCAGC
1101  GGCGGCGAAT AAGGGCAAGT GTCAAGATGG TGCGATCGGT GCGGCGGTCG
1151  GTGAAATCCT TGGCGAAACC CTACTGGACG GCAGAGACCC TGGCAGCCTG
1201  AATGTGAAGG ACAGGGCAAA AATCATTGCT AAGGCGAAGC TGGCAGCAGG
1251  GGCGGTTGCG GCGTTGAGTA AGGGGGATGT GAGTACGGCG GCGAATGCGG
```



```
1301  CTGCTGTGGC GGTAGAGAAT AATTCTTTAA ATGATATACA GGATCGTTTG
1351  TTGAGTGGAA ATTATGCTTT ATGTATGAGT GCAGGAGGAG CAGAAAGCTT
1401  TTGTGAGTCT TATCGACCAC TGGGCTTGCC ACACTTTGTA AGTGTTTCAG
1451  GAGAAATGAA ATTACCTAAT AAATTCGGGA ATCGTATGGT TAATGGAAAA
1501  TTAATTATTA ACACTAGAAA TGGCAATGTA TATTTCTCTG TAGGTAAAAT
1551  ATGGAGTACT GTAAAATCAA CAAAATCAAA TATAAGTGGG GTATCTGTCG
1601  GTTGGGTTTT AAATGTTTCC CCTAATGATT ATTTAAAAGA AGCATCTATG
1651  AATGATTTCA GAAATAGTAA TCAAAATAAA GCCTATGCAG AAATGATTTC
1701  CCAGACTTTG GTAGGTGAGA GTGTTGGTGG TAGTCTTTGT CTGACAAGAG
1751  CCTGCTTTTC GGTAAGTTCA ACAATATCTA AATCTAAATC TCCTTTTAAA
1801  GATTCAAAAA TTATTGGGGA AATCGGTTTG GGAAGTGGTG TTGCTGCAGG
1851  AGTAGAAAAA ACAATATACA TAGGTAACAT AAAAGATATT GATAAATTTA
1901  TTAGTGCAAA CATAAAAAAA TAG
```

This corresponds to the amino acid sequence <SEQ ID 20; ORF49-1>:

```
  1  MQLLAAEGIH QHQLNVQKST RFIGIKVGKS NYSKNELNET KLPVRVIAQT
 51  AKTRSGWDTV LEGTEFKTTL SGADIQAGVG EKARADAKII LKGIVNRIQT
101  EEKLESNSTV WQKQAGSGST VETLKLPSFE GPALPKLTAP GGYIADIPKG
151  NLKTEIEKLA KQPEYAYLKQ LQTVKDVNWN QVQLAYDKWD YKQEGLTGAG
201  AAIIALAVTV VTSGAGTGAV LGLNGAAAAA TDAAFASLAS QASVSFINNK
251  GNIGNTLKEL GRSSTVKNLM VAVATAGVAD KIGASALNNV SDKQWINNLT
301  VNLANAGSAA LINTAVNGGS LKDNLEANIL AALVNTAHGE AASKIKQLDQ
351  HYIAHKIAHA IAGCAAAAAN KGKCQDGAIG AAVGEILGET LLDGRDPGSL
401  NVKDRAKIIA KAKLAAGAVA ALSKGDVSTA ANAAAVAVEN NSLNDIQDRL
451  LSGNYALCMS AGGAESFCES YRPLGLPHFV SVSGEMKLPN KFGNRMVNGK
501  LIINTRNGNV YFSVGKIWST VKSTKSNISG VSVGWVLNVS PNDYLKEASM
551  NDFRNSNQNK AYAEMISQTL VGESVGGSLC LTRACFSVSS TISKSKSPFK
601  DSKIIGEIGL GSGVAAGVEK TIYIGNIKDI DKFISANIKK *
```

Computer analysis predicts a transmembrane domain and also indicates that ORF49 has no significant amino acid homology with known proteins. A corresponding ORF from *N.meningitidis* strain A was, however, identified:

ORF49 shows 86.1% identity over a 173aa overlap with an ORF (ORF49a) from strain A of *N. meningitidis*:

```
                                                         10        20        30
orf49.pep                                  GTEFKTTLSGADIQAGVGEKARADAKIILK
                                           ||||||||:||||||||| ||||:||||||
orf49a       SKNELNETKLPVRVVAQXAATRSGWDTVLEGTEFKTTLAGADIQAGVXEKARVDAKIILK
                     40        50        60        70        80        90

                     40        50        60        70        80        90
orf49.pep    GIVNRIQTEEKLESNSTVWQKQAGSGSTVETLKLPSFEGPALPKLTAPGGYIADIPKGNL
             |||||||:|||||:||||||||||| |||:||||||||||:|: |||:||||||:|||||||
orf49a       GIVNRIQSEEKLETNSTVWQKQAGRGSTIETLKLPSFESPTPPKLSAPGGYIVDIPKGNL
                    100       110       120       130       140       150

                    100       110       120       130       140       150
orf49.pep    KTEIEKLAKQPEYAYLKQLQTVKDVNWNQVQLAYDKWDYKQEGLTGAGAAIXALAVTVVT
             |||||||:||||||||||||||::|::|||||||||||||:|||||||||| ||||| |||||||
orf49a       KTEIEKLSKQPEYAYLKQLQVAKNINWNQVQLAYDRWDYKQEGLTEAGAAIIALAVTVVT
                    160       170       180       190       200       210

                    160       170
orf49.pep    SGAGTGAVLGLXRVAAAATDAAF
             |||||||||||   : |||||||
orf49a       SGAGTGAVLGLNGAXAAATDAAFASLASQASVSFINNKGDVGKTLKELGRSSTVKNLVVA
```

220 230 240 250 260 270

ORF49-1 and ORF49a show 83.2% identity in 457 aa overlap:

```
orf49a.pep  XQLLAEEGIHKHELDVQKSRRFIGIKVGXSNYSKNELNETKLPVRVVAQXAATRSGWDTV
orf49-1     MQLLAAEGIHQHQLNVQKSTRFIGIKVGKSNYSKNELNETKLPVRVIAQTAKTRSGWDTV

orf49a.pep  LEGTEFKTTLAGADIQAGVXEKARVDAKIILKGIVNRIQSEEKLETNSTVWQKQAGRGST
orf49-1     LEGTEFKTTLSGADIQAGVGEKARADAKIILKGIVNRIQTEEKLESNSTVWQKQAGSGST

orf49a.pep  IETLKLPSFESPTPPKLSAPGGYIVDIPKGNLKTEIEKLSKQPEYAYLKQLQVAKNINWN
orf49-1     VETLKLPSFEGPALPKLTAPGGYIADIPKGNLKTEIEKLAKQPEYAYLKQLQTVKDVNWN

orf49a.pep  QVQLAYDRWDYKQEGLTEAGAAIIALAVTVVTSGAGTGAVLGLNGAXAAATDAAFASLAS
orf49-1     QVQLAYDKWDYKQEGLTGAGAAIIALAVTVVTSGAGTGAVLGLNGAAAAATDAAFASLAS

orf49a.pep  QASVSFINNKGDVGKTLKELGRSSTVKNLVVAAATAGVADKIGASALXNVSDKQWINNLT
orf49-1     QASVSFINNKGNIGNTLKELGRSSTVKNLMVAVATAGVADKIGASALNNVSDKQWINNLT

orf49a.pep  VNLANAGSAALINTAVNGGSLKDXLEANILAALVNTAHGEAASKIKQLDQHYIVHKIAHA
orf49-1     VNLANAGSAALINTAVNGGSLKDNLEANILAALVNTAHGEAASKIKQLDQHYIAHKIAHA

orf49a.pep  IAGCAAAAANKGKCQDGAIGAAVGEIVGEALTHGKNPDTLTAKEREQILAYSKLVAGTVS
orf49-1     IAGCAAAAANKGKCQDGAIGAAVGEILGETLLDGRDPGSLNVKDRAKIIAKAKLAAGAVA

orf49a.pep  GVVGGDVNAAANAAEVAVKNNQLSDXEGREFDNEMTACAKQNXPQLCRKNTVKKYQNVAD
orf49-1     ALSKGDVSTAANAAAVAVENNSLNDIQDRLLSGNYALCMSAGGAESFCESYRPLGLPHFV

orf49a.pep  KRLAASIAICTDISRSTECRTIRKQHLIDSRSLHSSWEAGLIGKDDEWYKLFSKSYTQAD

orf49-1     SVSGEMKLPNKFGNRMVNGKLIINTRNGNVYFSVGKIWSTVKSTKSNISGVSVGWVLNVS
```

The complete length ORF49a nucleotide sequence <SEQ ID 21> is:

```
   1 NTGCAACTGC TGGCAGAAGA AGGCATCCAC AAGCACGAGT TGGATGTCCA
  51 AAAAAGCCGC CGCTTTATCG GCATCAAGGT AGGTNAGAGC AATTACAGTA
 101 AAAACGAACT GAACGAAACC AAATTGCCTG TCCGCGTCGT CGCCCAAANT
 151 GCAGCCACCC GTTCAGGCTG GGATACCGTG CTCGAAGGTA CCGAATTCAA
 201 AACCACGCTG GCCGGTGCCG ACATTCAGGC AGGTGTANGC GAAAAAGCCC
 251 GTGTCGATGC GAAAATTATC CTCAAAGGCA TTGTGAACCG TATCCAGTCG
 301 GAAGAAAAAT TAGAAACCAA CTCAACCGTA TGGCAGAAAC AGGCCGGACG
 351 CGGCAGCACT ATCGAAACGC TAAAACTGCC CAGCTTCGAA AGCCCTACTC
 401 CGCCCAAATT GTCCGCACCC GGCGGNTATA TCGTCGACAT TCCGAAAGGC
 451 AATCTGAAAA CCGAAATCGA AAAGCTGTCC AAACAGCCCG AGTATGCCTA
 501 TCTGAAACAG CTCCAAGTAG CGAAAAACAT CAACTGGAAT CAGGTGCAGC
 551 TTGCTTACGA CAGATGGGAC TACAAACAGG AGGGCTTAAC CGAAGCAGGT
 601 GCGGCGATTA TCGCACTGGC CGTTACCGTG GTCACCTCAG GCGCAGGAAC
 651 CGGAGCCGTA TTGGGATTAA ACGGTGCGNC CGCCGCCGCA ACCGATGCAG
 701 CATTCGCCTC TTTGGCCAGC CAGGCTTCCG TATCGTTCAT CAACAACAAA
 751 GGCGATGTCG GCAAAACCCT GAAAGAGCTG GGCAGAAGCA GCACGGTGAA
 801 AAATCTGGTG GTTGCCGCCG CTACCGCAGG CGTAGCCGAC AAAATCGGCG
 851 CTTCGGCACT GANCAATGTC AGCGATAAGC AGTGGATCAA CAACCTGACC
 901 GTCAACCTAG CCAATGCGGG CAGTGCCGCA CTGATTAATA CCGCTGTCAA
 951 CGGCGGCAGC CTGAAAGACA NTCTGGAAGC GAATATCCTT GCGGCTTTGG
1001 TCAATACCGC GCATGGAGAA GCAGCCAGTA AAATCAAACA GTTGGATCAG
```



```
1051 CACTACATAG TCCACAAGAT TGCCCATGCC ATAGCGGGCT GTGCGGCAGC
1101 GGCGGCGAAT AAGGGCAAGT GTCAGGATGG TGCGATAGGT GCGGCTGTGG
1151 GCGAGATAGT CGGGGAGGCT TTGACAAACG GCAAAAATCC TGACACTTTG
1201 ACAGCTAAAG AACGCGAACA GATTTTGGCA TACAGCAAAC TGGTTGCCGG
1251 TACGGTAAGC GGTGTGGTCG GCGGCGATGT AAATGCGGCG GCGAATGCGG
1301 CTGAGGTAGC GGTGAAAAAT AATCAGCTTA GCGACNAAGA GGGTAGAGAA
1351 TTTGATAACG AAATGACTGC ATGCGCCAAA CAGAATANTC CTCAACTGTG
1401 CAGAAAAAAT ACTGTAAAAA AGTATCAAAA TGTTGCTGAT AAAAGACTTG
1451 CTGCTTCGAT TGCAATATGT ACGGATATAT CCCGTAGTAC TGAATGTAGA
1501 ACAATCAGAA AACAACATTT GATCGATAGT AGAAGCCTTC ATTCATCTTG
1551 GGAAGCAGGT CTAATTGGTA AAGATGATGA ATGGTATAAA TTATTCAGCA
1601 AATCTTACAC CCAAGCAGAT TTGGCTTTAC AGTCTTATCA TTTGAATACT
1651 GCTGCTAAAT CTTGGCTTCA ATCGGGCAAT ACAAAGCCTT TATCCGAATG
1701 GATGTCCGAC CAAGGTTATA CACTTATTTC AGGAGTTAAT CCTAGATTCA
1751 TTCCAATACC AAGAGGGTTT GTAAAACAAA ATACACCTAT TACTAATGTC
1801 AAATACCCGG AAGGCATCAG TTTCGATACA AACCTANAAA GACATCTGGC
1851 AAATGCTGAT GGTTTTAGTC AAGAACAGGG CATTAAAGGA GCCCATAACC
1901 GCACCAATNT TATGGCAGAA CTAAATTCAC GAGGAGGANG NGTAAAATCT
1951 GAAACCCANA CTGATATTGA AGGCATTACC CGAATTAAAT ATGAGATTCC
2001 TACACTAGAC AGGACAGGTA AACCTGATGG TGGATTTAAG GAAATTTCAA
2051 GTATAAAAAC TGTTTATAAT CCTAAAAANT TTTNNGATGA TAAAATACTT
2101 CAAATGGCTC AANATGCTGN TTCACAAGGA TATTCAAAAG CCTCTAAAAT
2151 TGCTCAAAAT GAAAGAACTA AATCAATATC GGAAAGAAAA AATGTCATTC
2201 AATTCTCAGA AACCTTTGAC GGAATCAAAT TTAGANNNTA TNTNGATGTA
2251 AATACAGGAA GAATTACAAA CATTCACCCA GAATAATTTA A
```

This encodes a protein having amino acid sequence <SEQ ID 22>:

```
  1 XQLLAEEGIH KHELDVQKSR RFIGIKVGXS NYSKNELNET KLPVRVVAQX
 51 AATRSGWDTV LEGTEFKTTL AGADIQAGVX EKARVDAKII LKGIVNRIQS
101 EEKLETNSTV WQKQAGRGST IETLKLPSFE SPTPPKLSAP GGYIVDIPKG
151 NLKTEIEKLS KQPEYAYLKQ LQVAKNINWN QVQLAYDRWD YKQEGLTEAG
201 AAIIALAVTV VTSGAGTGAV LGLNGAXAAA TDAAFASLAS QASVSFINNK
251 GDVGKTLKEL GRSSTVKNLV VAAATAGVAD KIGASALXNV SDKQWINNLT
301 VNLANAGSAA LINTAVNGGS LKDXLEANIL AALVNTAHGE AASKIKQLDQ
351 HYIVHKIAHA IAGCAAAAAN KGKCQDGAIG AAVGEIVGEA LTNGKNPDTL
401 TAKEREQILA YSKLVAGTVS GVVGGDVNAA ANAAEVAVKN NQLSDXEGRE
451 FDNEMTACAK QNXPQLCRKN TVKKYQNVAD KRLAASIAIC TDISRSTECR
501 TIRKQHLIDS RSLHSSWEAG LIGKDDEWYK LFSKSYTQAD LALQSYHLNT
551 AAKSWLQSGN TKPLSEWMSD QGYTLISGVN PRFIPIPRGF VKQNTPITNV
601 KYPEGISFDT NLXRHLANAD GFSQEQGIKG AHNRTNXMAE LNSRGGXVKS
651 ETXTDIEGIT RIKYEIPTLD RTGKPDGGFK EISSIKTVYN PKXFXDDKIL
701 QMAQXAXSQG YSKASKIAQN ERTKSISERK NVIQFSETFD GIKFRXYXDV
751 NTGRITNIHP E*
```

Based on the presence of a putative transmembrane domain, it is predicted that these proteins from *N.meningitidis*, and their epitopes, could be useful antigens for vaccines or diagnostics.

Example 5

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 23>

```
  1 ..CGGATCGTTG TAGGTTTGCG GATTTCTTGC GCCGTAGTCA CCGTAGTCCC
 51   AAGTATAACC CAAGGCTTTG TCTTCGCCTT TCATTCCGAT AAGGGATATG
101   ACGCTTTGGT CGGTATAGCC GTCTTGGGAA CCTTTGTCCA CCCAACGCAT
151   ATCTGCCTGC GGATTCTCAT TGCCGCTTCT TGGCTGCTGA TTTTTCTGCC
201   TTCGCGTTTT TCAACTTCGC GCTTGAGGGC TTCGGCATAT TTGTCGGCCA
251   ACGCCATTTC TTTCGGATGC AGCTGCCTAT TGTTCCAATC TACATTCGCA
301   CCCACCACAG CACCACCACT ACCACCAGTT GCATAG
```

This corresponds to the amino acid sequence <SEQ ID 24; ORF50>:

```
  1  ..RIVVGLRISC AVVTVVPSIT QGFVFAFHSD KGYDALVGIA VLGTFVHPTH
 51    ICLRILIAAS WLLIFLPSRF STSRLRASAY LSANAISFGC SCLLFQSTFA
101    PTTAPPLPPV A*
```

Computer analysis predicts two transmembrane domains and also indicates that ORF50 has no significant amino acid homology with known proteins.

Based on the presence of a putative transmembrane domain, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 6

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 25>

```
   1  ..AAGTTTGACT TTACCTGGTT TATTCCGGCG GTAATCAAAT ACCGCCGGTT
  51    GTTTTTTGAA GTATTGGTGG TGTCGGTGGT GTTGCAGCTG TTTGCGCTGA
 101    TTACGCCTCT GTTTTTCCAA GTGGTGATGG ACAAGGTGCT GGTACATCGG
 151    GGATTCTCTA CTTTGGATGT GGTGTCGGTG GCTTTGTTGG TGGTGTCGCT
 201    GTTTGAGATT GTGTTGGGCG GTTTGCGGAC GTATCTGTTT GCACATACGA
 251    CTTCACGTAT TGATGTGGAA TTGGGCGCGC GTTTGTTCCG GCATCTGCTT
 301    TCCCTGCCTT TATCCTATTT CGAGCACAGA CGAGTGGGTG ATACGGTGGC
 351    TCGGGTGCGG GAATTGGAGC AGATTCGCAA TTTCTTGACC GGTCAGGCGC
 401    TGACTTCGGT GTTGGATTTG GCGTTTTCGT TTATCTTTCT GGCGGTGATG
 451    TGGTATTACA GCTCCACTCT GACTTGGGTG GTATTGGCTT CGTTG.....
                                  //
1451    .......... .......... .......... .......... ..........
1501    .......... .......... .......... .......... ..ATTTGCGC
1551    CAACCGGACG GTGCTGATTA TCGCCCACCG TCTGTCCACT GTTAAAACGG
1601    CACACCGGAT CATTGCCATG GATAAAGGCA GGATTGTGGA AGCGGGAACA
1651    CAGCAGGAAT TGCTGGCGAA CG..AACGGA TATTACCGCT ATCTGTATGA
1701    TTTACAGAAC GGGTAG
```

This corresponds to the amino acid sequence <SEQ ID 26; ORF39>:

```
  1  ..KFDFTWFIPA VIKYRRLFFE VLVVSVVLQL FALITPLFFQ VVMDKVLVHR
 51    GFSTLDVVSV ALLVVSLFEI VLGGLRTYLF AHTTSRIDVE LGARLFRHLL
101    SLPLSYFEHR RVGDTVARVR ELEQIRNFLT GQALTSVLDL AFSFIFLAVM
151    WYYSSTLTWV VLASL..... .......... .......... ..........
                               //
501    .......... ....ICANRT VLIIAHRLST VKTAHRIIAM DKGRIVEAGT
551    QQELLANXNG YYRYLYDLQN G*
```

Further work revealed the complete nucleotide sequence <SEQ ID 27>:

```
  1  ATGTCTATCG TATCCGCACC GCTCCCCGCC CTTTCCGCCC TCATCATCCT
 51  CGCCCATTAC CACGGCATTG CCGCCAATCC TGCCGATATA CAGCATGAAT
101  TTTGTACTTC CGCACAGAGC GATTTAAATG AAACGCAATG GCTGTTAGCC
151  GCCAAATCTT TGGGATTGAA GGCAAAGGTA GTCCGCCAGC CTATTAAACG
201  TTTGGCTATG GCGACTTTAC CCGCATTGGT ATGGTGTGAT GACGGCAACC
251  ATTTCATTTT GGCCAAAACA GACGGTGAGG GTGAGCATGC CCAATTTTTG
301  ATACAGGATT TGGTTACGAA TAAGTCTGCG GTATTGTCTT TTGCCGAATT
351  TTCTAACAGA TATTCGGGCA AACTGATATT GGTTGCTTCC CGCGCTTCGG
401  TATTGGGCAG TTTGGCAAAG TTTGACTTTA CCTGGTTTAT TCCGGCGGTA
451  ATCAAATACC GCCGGTTGTT TTTTGAAGTA TTGGTGGTGT CGGTGGTGTT
501  GCAGCTGTTT GCGCTGATTA CGCCTCTGTT TTTCCAAGTG GTGATGGACA
```



```
 551  AGGTGCTGGT ACATCGGGGA TTCTCTACTT TGGATGTGGT GTCGGTGGCT
 601  TTGTTGGTGG TGTCGCTGTT TGAGATTGTG TTGGGCGGTT TGCGGACGTA
 651  TCTGTTTGCA CATACGACTT CACGTATTGA TGTGGAATTG GGCGCGCGTT
 701  TGTTCCGGCA TCTGCTTTCC CTGCCTTTAT CCTATTTCGA GCACAGACGA
 751  GTGGGTGATA CGGTGGCTCG GGTGCGGGAA TTGGAGCAGA TTCGCAATTT
 801  CTTGACCGGT CAGGCGCTGA CTTCGGTGTT GGATTTGGCG TTTTCGTTTA
 851  TCTTTCTGGC GGTGATGTGG TATTACAGCT CCACTCTGAC TTGGGTGGTA
 901  TTGGCTTCGT TGCCTGCCTA TGCGTTTTGG TCGGCATTTA TCAGTCCGAT
 951  ACTGCGGACG CGTCTGAACG ATAAGTTCGC GCGCAATGCA GACAACCAGT
1001  CGTTTTTAGT AGAAAGCATC ACTGCGGTGG GTACGGTAAA GGCGATGGCG
1051  GTGGAGCCGC AGATGACGCA GCGTTGGGAC AATCAGTTGG CGGCTTATGT
1101  GGCTTCGGGA TTTCGGGTAA CGAAGTTGGC GGTGGTCGGC CAGCAGGGGG
1151  TGCAGCTGAT TCAGAAGCTG GTGACGGTGG CGACGTTGTG GATTGGCGCA
1201  CGGCTGGTAA TTGAGAGCAA GCTGACGGTG GGGCAGCTGA TTGCGTTTAA
1251  TATGCTCTCG GGACAGGTGG CGGCGCCTGT TATCCGTTTG GCGCAGTTGT
1301  GGCAGGATTT CCAGCAGGTG GGGATTTCGG TGGCGCGTTT GGGGGATATT
1351  CTGAATGCGC CGACCGAGAA TGCGTCTTCG CATTTGGCTT TGCCCGATAT
1401  CCGGGGGGAG ATTACGTTCG AACATGTCGA TTTCCGCTAT AAGGCGGACG
1451  GCAGGCTGAT TTTGCAGGAT TTGAACCTGC GGATTCGGGC GGGGGAAGTG
1501  CTGGGGATTG TGGGACGTTC GGGGTCGGGC AAATCCACAC TCACCAAATT
1551  GGTGCAGCGT CTGTATGTAC CGGAGCAGGG ACGGGTGTTG GTGGACGGCA
1601  ACGATTTGGC TTTGGCCGCT CCTGCCTGGC TGCGGCGGCA GGTCGGCGTG
1651  GTCTTGCAGG AGAATGTGCT GCTCAACCGC AGCATACGCG ACAATATCGC
1701  GCTGACGGAT ACGGGTATGC CGCTGGAACG CATTATCGAA GCAGCCAAAC
1751  TGGCGGGCGC ACACGAGTTT ATTATGGAGC TGCCGGAAGG CTACGGCACC
1801  GTGGTGGGCG AACAAGGGGC CGGCTTGTCG GGCGGACAGC GGCAGCGTAT
1851  TGCGATTGCC CGCGCGTTAA TCACCAATCC GCGCATTCTG ATTTTTGATG
1901  AAGCCACCAG CGCGCTGGAT TATGAAAGTG AACGAGCGAT TATGCAGAAC
1951  ATGCAGGCCA TTTGCGCCAA CCGGACGGTG CTGATTATCG CCCACCGTCT
2001  GTCCACTGTT AAAACGGCAC ACCGGATCAT TGCCATGGAT AAAGGCAGGA
2051  TTGTGGAAGC GGGAACACAG CAGGAATTGC TGGCGAAGCC GAACGGATAT
2101  TACCGCTATC TGTATGATTT ACAGAACGGG TAG
```

This corresponds to the amino acid sequence <SEQ ID 28; ORF39-1>:

```
  1  MSIVSAPLPA LSALIILAHY HGIAANPADI QHEFCTSAQS DLNETQWLLA
 51  AKSLGLKAKV VRQPIKRLAM ATLPALVWCD DGNHFILAKT DGEGEHAQFL
101  IQDLVTNKSA VLSFAEFSNR YSGKLILVAS RASVLGSLAK FDFTWFIPAV
151  IKYRRLFFEV LVVSVVLQLF ALITPLFFQV VMDKVLVHRG FSTLDVVSVA
201  LLVVSLFEIV LGGLRTYLFA HTTSRIDVEL GARLFRHLLS LPLSYFEHRR
251  VGDTVARVRE LEQIRNFLTG QALTSVLDLA FSFIFLAVMW YYSSTLTWVV
301  LASLPAYAFW SAFISPILRT RLNDKFARNA DNQSFLVESI TAVGTVKAMA
351  VEPQMTQRWD NQLAAYVASG FRVTKLAVVG QQGVQLIQKL VTVATLWIGA
401  RLVIESKLTV GQLIAFNMLS GQVAAPVIRL AQLWQDFQQV GISVARLGDI
451  LNAPTENASS HLALPDIRGE ITFEHVDFRY KADGRLILQD LNLRIRAGEV
501  LGIVGRSGSG KSTLTKLVQR LYVPEQGRVL VDGNDLALAA PAWLRRQVGV
551  VLQENVLLNR SIRDNIALTD TGMPLERIIE AAKLAGAHEF IMELPEGYGT
601  VVGEQGAGLS GGQRQRIAIA RALITNPRIL IFDEATSALD YESERAIMQN
651  MQAICANRTV LIIAHRLSTV KTAHRIIAMD KGRIVEAGTQ QELLAKPNGY
701  YRYLYDLQNG *
```

Computer analysis of this amino acid sequence gave the following results:

<u>Homology with a predicted ORF from *N.meningitidis* (strain A)</u>

ORF39 shows 100% identity over a 165aa overlap with an ORF (ORF39a) from strain A of *N. meningitidis*:

```
                        10        20        30
orf39.pep     KFDFTWFIPAVIKYRRLFFEVLVVSVVLQL
              ||||||||||||||||||||||||||||||
```

```
orf39a      AVLSFAEFSNRYSGKLILVASRASVLGSLAKFDFTWFIPAVIKYRRLFFEVLVVSVVLQL
        110       120       130       140       150       160

                 40        50        60        70        80        90
orf39.pep   FALITPLFFQVVMDKVLVHRGFSTLDVVSVALLVVSLFEIVLGGLRTYLFAHTTSRIDVE
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a      FALITPLFFQVVMDKVLVHRGFSTLDVVSVALLVVSLFEIVLGGLRTYLFAHTTSRIDVE
        170       180       190       200       210       220

                100       110       120       130       140       150
orf39.pep   LGARLFRHLLSLPLSYFEHRRVGDTVARVRELEQIRNFLTGQALTSVLDLAFSFIFLAVM
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a      LGARLFRHLLSLPLSYFEHRRVGDTVARVRELEQIRNFLTGQALTSVLDLAFSFIFLAVM
        230       240       250       260       270       280.

                160       170       180       190       200       210
orf39.pep   WYYSSTLTWVVLASLXXXXXXXXXXXXXXXXXXXXXXXXXXXICANRTVLIIAHRLSTV
            |||||||||||||||
orf39a      WYYSSTLTWVVLASLPAYAFWSAFISPILRTRLNDKFARNADNQSFLVESITAVGTVKAM
        290       300       310       320       330       340
```

ORF39-1 and ORF39a show 99.4% identity in 710 aa overlap:

```
orf39-1.pep   MSIVSAPLPALSALIIILAHYHGIAANPADIQHEFCTSAQSDLNETQWLLAAKSLGLKAKV
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a        MSIVSAPLPALSALIIILAHYHGIAANPADIQHEFCTSAQSDLNETQWLLAAKSLGLKAKV

orf39-1.pep   VRQPIKRLAMATLPALVWCDDGNHFILAKTDGEGEHAQFLIQDLVTNKSAVLSFAEFSNR
              |||||||||||||||||||||||||||||||| |||||:|||||:|||||||||||||||
orf39a        VRQPIKRLAMATLPALVWCDDGNHFILAKTDGGGEHAQYLIQDLTTNKSAVLSFAEFSNR

orf39-1.pep   YSGKLILVASRASVLGSLAKFDFTWFIPAVIKYRRLFFEVLVVSVVLQLFALITPLFFQV
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a        YSGKLILVASRASVLGSLAKFDFTWFIPAVIKYRRLFFEVLVVSVVLQLFALITPLFFQV

orf39-1.pep   VMDKVLVHRGFSTLDVVSVALLVVSLFEIVLGGLRTYLFAHTTSRIDVELGARLFRHLLS
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a        VMDKVLVHRGFSTLDVVSVALLVVSLFEIVLGGLRTYLFAHTTSRIDVELGARLFRHLLS

orf39-1.pep   LPLSYFEHRRVGDTVARVRELEQIRNFLTGQALTSVLDLAFSFIFLAVMWYYSSTLTWVV
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a        LPLSYFEHRRVGDTVARVRELEQIRNFLTGQALTSVLDLAFSFIFLAVMWYYSSTLTWVV

orf39-1.pep   LASLPAYAFWSAFISPILRTRLNDKFARNADNQSFLVESITAVGTVKAMAVEPQMTQRWD
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a        LASLPAYAFWSAFISPILRTRLNDKFARNADNQSFLVESITAVGTVKAMAVEPQMTQRWD

orf39-1.pep   NQLAAYVASGFRVTKLAVVGQQGVQLIQKLVTVATLWIGARLVIESKLTVGQLIAFNMLS
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a        NQLAAYVASGFRVTKLAVVGQQGVQLIQKLVTVATLWIGARLVIESKLTVGQLIAFNMLS

orf39-1.pep   GQVAAPVIRLAQLWQDFQQVGISVARLGDILNAPTENASSHLALPDIRGEITFEHVDFRY
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a        GQVAAPVIRLAQLWQDFQQVGISVARLGDILNAPTENASSHLALPDIRGEITFEHVDFRY

orf39-1.pep   KADGRLILQDLNLRIRAGEVLGIVGRSGSGKSTLTKLVQRLYVPEQGRVLVDGNDLALAA
              ||||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||
orf39a        KADGRLILQDLNLRIRAGEVLGIVGRSGSGKSTLTKLVQRLYVPAQGRVLVDGNDLALAA

orf39-1.pep   PAWLRRQVGVVLQENVLLNRSIRDNIALTDTGHPLERIIEAAKLAGAHEFIMELPEGYGT
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a        PAWLRRQVGVVLQENVLLNRSIRDNIALTDTGHPLERIIEAAKLAGAHEFIMELPEGYGT

orf39-1.pep   VVGEQGAGLSGGQRQRIAIARALITNPRILIFDEATSALDYESERAIMQNMQAICANRTV
```



```
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a        VVGEQGAGLSGGQRQRIAIARALITNPRILIFDEATSALDYESERAIMQNMQAICANRTV

orf39-1.pep   LIIAHRLSTVKTAHRIIAMDKGRIVEAGTQQELLAKPNGYYRYLYDLQNGX
              |||||||||||||||||||||||||||||||||||||||||||||||||||
orf39a        LIIAHRLSTVKTAHRIIAMDKGRIVEAGTQQELLAKPNGYYRYLYDLQNGX
```

The complete length ORF39a nucleotide sequence <SEQ ID 29> is:

```
   1  ATGTCTATCG TATCCGCACC GCTCCCCGCC CTTTCCGCCC TCATCATCCT
  51  CGCCCATTAC CACGGCATTG CCGCCAATCC TGCCGATATA CAGCATGAAT
 101  TTTGTACTTC CGCACAGAGC GATTTAAATG AAACGCAATG GCTGTTAGCC
 151  GCCAAATCTT TGGGATTGAA GGCAAAGGTA GTCCGCCAGC CTATTAAACG
 201  TTTGGCTATG GCGACTTTAC CCGCATTGGT ATGGTGTGAT GACGGCAACC
 251  ATTTTATTTT GGCTAAAACA GACGGTGGGG GTGAGCATGC CCAATATCTA
 301  ATACAGGATT TAACTACGAA TAAGTCTGCG GTATTGTCTT TTGCCGAATT
 351  TTCTAACAGA TATTCGGGCA AACTGATATT GGTTGCTTCC CGCGCTTCGG
 401  TATTGGGCAG TTTGGCAAAG TTTGACTTTA CCTGGTTTAT TCCGGCGGTA
 451  ATCAAATACC GCCGGTTGTT TTTTGAAGTA TTGGTGGTGT CGGTGGTGTT
 501  GCAGCTGTTT GCGCTGATTA CGCCTCTGTT TTTCCAAGTG GTGATGGACA
 551  AGGTGCTGGT ACATCGGGGA TTCTCTACTT TGGATGTGGT GTCGGTGGCT
 601  TTGTTGGTGG TGTCGCTGTT TGAGATTGTG TTGGGCGGTT TGCGGACGTA
 651  TCTGTTTGCA CATACGACTT CACGTATTGA TGTGGAATTG GGCGCGCGTT
 701  TGTTCCGGCA TCTGCTTTCC CTGCCTTTAT CCTATTTCGA GCACAGACGA
 751  GTGGGTGATA CGGTGGCTCG GGTGCGGGAA TTGGAGCAGA TTCGCAATTT
 801  CTTGACCGGT CAGGCGCTGA CTTCGGTGTT GGATTTGGCG TTTTCGTTTA
 851  TCTTTCTGGC GGTGATGTGG TATTACAGCT CCACTCTGAC TTGGGTGGTA
 901  TTGGCTTCGT TGCCTGCCTA TGCGTTTTGG TCGGCATTTA TCAGTCCGAT
 951  ACTGCGGACG CGTCTGAACG ATAAGTTCGC GCGCAATGCA GACAACCAGT
1001  CGTTTTTAGT AGAAAGCATC ACTGCGGTGG GTACGGTAAA GGCGATGGCG
1051  GTGGAGCCGC AGATGACGCA GCGTTGGGAC AATCAGTTGG CGGCTTATGT
1101  GGCTTCGGGA TTTCGGGTAA CGAAGTTGGC GGTGGTCGGC CAGCAGGGGG
1151  TGCAGCTGAT TCAGAAGCTG GTGACGGTGG CGACGTTGTG GATTGGCGCA
1201  CGGCTGGTAA TTGAGAGCAA GCTGACGGTG GGGCAGCTGA TTGCGTTTAA
1251  TATGCTCTCG GGACAGGTGG CGGCGCCTGT TATCCGTTTG GCGCAGTTGT
1301  GGCAGGATTT CCAGCAGGTG GGGATTTCGG TGGCGCGTTT GGGGGATATT
1351  CTGAATGCGC CGACCGAGAA TGCGTCTTCG CATTTGGCTT TGCCCGATAT
1401  CCGGGGGGAG ATTACGTTCG AACATGTCGA TTTCCGCTAT AAGGCGGACG
1451  GCAGGCTGAT TTTGCAGGAT TTGAACCTGC GGATTCGGGC GGGGGAAGTG
1501  CTGGGGATTG TGGGACGTTC GGGGTCGGGC AAATCCACAC TCACCAAATT
1551  GGTGCAGCGT CTGTATGTAC CGGCGCAGGG ACGGGTGTTG GTGGACGGCA
1601  ACGATTTGGC TTTGGCCGCT CCTGCTTGGC TGCGGCGGCA GGTCGGCGTG
1651  GTCTTGCAGG AGAATGTGCT GCTCAACCGC AGCATACGCG ACAATATCGC
1701  GCTGACGGAT ACGGGTATGC CGCTGGAACG CATTATCGAA GCAGCCAAAC
1751  TGGCGGGCGC ACACGAGTTT ATTATGGAGC TGCCGGAAGG CTACGGCACC
1801  GTGGTGGGCG AACAAGGGGC CGGCTTGTCG GGCGGACAGC GGCAGCGTAT
1851  TGCGATTGCC CGCGCGTTAA TCACCAATCC GCGCATTCTG ATTTTTGATG
1901  AAGCCACCAG CGCGCTGGAT TATGAAAGTG AACGAGCGAT TATGCAGAAC
1951  ATGCAGGCCA TTTGCGCCAA CCGGACGGTG CTGATTATCG CCCACCGTCT
2001  GTCCACTGTT AAAACGGCAC ACCGGATCAT TGCCATGGAT AAAGGCAGGA
2051  TTGTGGAAGC GGGAACACAG CAGGAATTGC TGGCGAAGCC GAACGGATAT
2101  TACCGCTATC TGTATGATTT ACAGAACGGG TAG
```

This encodes a protein having amino acid sequence <SEQ ID 30>:

```
  1  MSIVSAPLPA LSALIIILAHY HGIAANPADI QHEFCTSAQS DLNETQWLLA
 51  AKSLGLKAKV VRQPIKRLAM ATLPALVWCD DGNHFILAKT DGGGEHAQYL
101  IQDLTTNKSA VLSFAEFSNR YSGKLILVAS RASVLGSLAK FDFTWFIPAV
151  IKYRRLFFEV LVVSVVLQLF ALITPLFFQV VMDKVLVHRG FSTLDVVSVA
201  LLVVSLFEIV LGGLRTYLFA HTTSRIDVEL GARLFRHLLS LPLSYFEHRR
251  VGDTVARVRE LEQIRNFLTG QALTSVLDLA FSFIFLAVMW YYSSTLTWVV
301  LASLPAYAFW SAFISPILRT RLNDKFARNA DNQSFLVESI TAVGTVKAMA
351  VEPQMTQRWD NQLAAYVASG FRVTKLAVVG QQGVQLIQKL VTVATLWIGA
```

```
401  RLVIESKLTV GQLIAFNMLS GQVAAPVIRL AQLWQDFQQV GISVARLGDI
451  LNAPTENASS HLALPDIRGE ITFEHVDFRY KADGRLILQD LNLRIRAGEV
501  LGIVGRSGSG KSTLTKLVQR LYVPAQGRVL VDGNDLALAA PAWLRRQVGV
551  VLQENVLLNR SIRDNIALTD TGMPLERIIE AAKLAGAHEF IHELPEGYGT
601  VVGEQGAGLS GGQRQRIAIA RALITNPRIL IFDEATSALD YESERAIMQN
651  MQAICANRTV LIIAHRLSTV KTAHRIIAMD KGRIVEAGTQ QELLAKPNGY
701  YRYLYDLQNG *
```

ORF39a is homologous to a cytolysin from *A.pleuropneumoniae*:

```
sp|P26760|RT1B_ACTPL RTX-I TOXIN DETERMINANT B (TOXIN RTX-1 SECRETION ATP-
BINDING PROTEIN) (APX-IB) (HLY-IB) (CYTOLYSIN IB) (CLY-IB)
>gi|97137|pir||D43599 cytolysin IB - Actinobacillus pleuropneumoniae (serotype 9)
>gi|38944 (X61112) ClyI-B protein (Actinobacillus pleuropneumoniae) Length = 707
 Score =  931 bits (2379), Expect = 0.0
 Identities = 472/690 (68%), Positives = 540/690 (77%), Gaps = 3/690 (0%)

Query: 20  YHGIAANPADIQHEFCTSAQSDLNETQWXXXXXXXXXXXXVVRQPIKRLAMATLPALVWC 79
            YH IA NP +++H+F    + L+ T W            V++ I RLA   LPALVW
Sbjct: 20  YHNIAVNPEELKHKFDLEGKG-LDLTAWLLAAKSLELKAKQVKKAIDRLAFIALPALVWR 78

Query: 80  DDGNHFILAKTDGGGEHAQYLIQDLTTNKSAVLSFAEFSNRYSGKLILVASRASVLGSLA 139
            +DG HFIL K D   E  +YLI DL T+   +L  AEF + Y GKLILVASRAS++G LA
Sbjct: 79  EDGKHFILTKIDN--EAKKYLIFDLETHNPRILEQAEFESLYQGKLILVASRASIVGKLA 136

Query: 140 KFDFTWFIPAVIKYRRXXXXXXXXXXXXXXXXXITPLFFQVVMDKVLVHRGFXXXXXXXX 199
            KFDFTWFIPAVIKYR+                 ITPLFFQVVMDKVLVHRGF
Sbjct: 137 KFDFTWFIPAVIKYRKIFIETLIVSIFLQIFALITPLFFQVVMDKVLVHRGFSTLNVITV 196

Query: 200 XXXXXXXFEIVLGGLRTYLFAHTTSRIDVELGARLFRHLLSLPLSYFENRRVGDTVARVR 259
                   FEIVL GLRTY+FAH+TSRIDVELGARLFRHLL+LP+SYFE+RRVGDTVARVR
Sbjct: 197 ALAIVVLFEIVLNGLRTYIFAHSTSRIDVELGARLFRHLLALPISYFENRRVGDTVARVR 256

Query: 260 ELEQIRNFLTGQALTSVLDLAFSFIFLAVMWYYSSTLTWVVLASLPAYAFWSAFISPILR 319
            EL+QIRNFLTGQALTSVLDL FSFIF AVMWYYS  LT V+L SLP Y  WS FISPILR
Sbjct: 257 ELDQIRNFLTGQALTSVLDLMFSFIFFAVMWYYSPKLTLVILGSLPFYMGWSIFISPILR 316

Query: 320 TRLNDKFARNADNQSFLVESITAVGTVKAMAVEPQMTQRWDNQLAAYVASGFRVTKLAVV 379
            RL++KFAR ADNQSFLVES+TA+ T+KA+AV PQMT  WD QLA+YV++GFRVT LA +
Sbjct: 317 RRLDEKFARGADNQSFLVESVTAINTIKALAVTPQMTNTWDKQLASYVSAGFRVTTLATI 376

Query: 380 GQQGVQLIQKLVTVATLWIGARLVIESKLTVGQLIAFNMLSGQVAAPVIRLAQLWQDFQQ 439
            GQQGVQ IQK+V V TLW+GA LVI    L++GQLIAFNMLSGQV APVIRLAQLWQDFQQ
Sbjct: 377 GQQGVQFIQKVVMVITLWLGAHLVISGDLSIGQLIAFNMLSGQVIAPVIRLAQLWQDFQQ 436

Query: 440 VGISVARLGDILNAPTENASSHLALPDIRGEITFEHVDFRYKADGRLILQDLNLRIRAGE 499
            VGISV RLGD+LN+PTE+    LALP+I+G+ITF ++ FRYK D  +IL D+NL I+ GE
Sbjct: 437 VGISVTRLGDVLNSPTESYQGKLALPEIKGDITFRNIRFRYKPDAPVILNDVNLSIQQGE 496

Query: 500 VLGIVGRSGSGKSTLTKLVQRLYVPAQGRVLVDGNDLALAAPAWLRRQVGVVLQENVLLN 559
            V+GIVGRSGSGKSTLTKL+QR Y+P  G+VL+DG+DLALA P WLRRQVGVVLQ+NVLLN
Sbjct: 497 VIGIVGRSGSGKSTLTKLIQRFYIPENGQVLIDGHDLALADPNWLRRQVGVVLQDNVLLN 556

Query: 560 RSIRDNIALTDTGMPLERIIEAAKLAGAHEFIMELPEGYGTVVGEQGAGLSGGQRQRIAI 619
            RSIRDNIAL D GMP+E+I+ AAKLAGAHEFI EL EGY T+VGEQGAGLSGGQRQRIAI
Sbjct: 557 RSIRDNIALADPGMPMEKIVHAAKLAGAHEFISELREGYNTIVGEQGAGLSGGQRQRIAI 616

Query: 620 ARALITNPRILIFDEATSALDYESERAIMQNMQAICANRTVLIIAHRLSTVKTAHRIIAM 679
            ARAL+ NP+ILIFDEATSALDYESE  IM+NM  IC  RTV+IIAHRLSTVK A RII M
Sbjct: 617 ARALVNNPKILIFDEATSALDYESEHIIMRNMHQICKGRTVIIIAHRLSTVKNADRIIVM 676

Query: 680 DKGRIVEAGTQQELLAKPNGYYRYLYDLQN 709
            +KG+IVE G  +ELLA PNG Y YL+ LQ+
Sbjct: 677 EKGQIVEQGKHKELLADPNGLYHYLHQLQS 706
```


Homology with the HlyB leucotoxin secretion ATP-binding protein of *Haemophilus actinomycetemcomitans* (accession number X53955)

ORF39 and HlyB protein show 71% and 69% amino acid identity in 167 and 55 overlap at the N- and C-terminal regions, respectively:

```
Orf39   1   KFDFTWFIPAVIKYRRXXXXXXXXXXXXXXXXXITPLFFQVVMDKVLVHRGFXXXXXXXX 60
            KFDFTWFIPAVIKYR+                 ITPLFFQVVMDKVLVHRGF
HlyB    137 KFDFTWFIPAVIKYRKIFIETLIVSIFLQIFALITPLFFQVVMDKVLVHRGFSTLNVITV 196

Orf39   61  XXXXXXXFEIVLGGLRTYLFAHTTSRIDVELGARLFRHLLSLPLSYFENRRVGDTVARVR 120
                   FEI+LGGLRTY+FAH+TSRIDVELGARLFRHLL+LP+SYFE RRVGDTVARVR
HlyB    197 ALAIVVLFEIILGGLRTYVFAHSTSRIDVELGARLFRHLLALPISYFEARRVGDTVARVR 256

Orf39   121 ELEQIRNFLTGQALTSVLDLAFSFIFLAVMWYYSSTLTWVVLASLIC 167
            EL+QIRNFLTGQALTS+LDL FSFIF AVMWYYS  LT VVL SL C
HlyB    257 ELDQIRNFLTGQALTSILDLLFSFIFFAVMWYYSPKLTLVVLGSLPC 303

                              //

Orf39   166 ICANRTVLIIAHRLSTVKTAHRIIAMDKGRIVEAGTQQELLANXNGYYRYLYDLQ 220
            IC NRTVLIIAHRLSTVK A RII MDKG I+E G  QELL +  G Y YL+ LQ
HlyB    651 ICQNRTVLIIAHRLSTVKNADRIIVMDKGEIIEQGKHQELLKDEKGLYSYLHQLQ 705
```

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 7

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 31>

```
  1  ATGAAATACT TGATCCGCAC CGCCTTACTC GCAGTCGCAG CCGCCGGCAT
 51  CTACGCCTGC CAACCGCAAT CCGAAGCCGC AGTGCAAGTC AAGGCTGAAA
101  ACAGCCTGAC CGCTATGCGC TTAGCCGTCG CCGACAAACA GGCAGAGATT
151  GACGGGTTGA ACGCCCAAAk sGACGCCGAA ATCAGA...
```

This corresponds to the amino acid sequence <SEQ ID 32; ORF52>:

```
 1  MKYLIRTALL AVAAAGIYAC QPQSEAAVQV KAENSLTAMR LAVADKQAEI
51  DGLNAQXDAE IR..
```

Further work revealed the complete nucleotide sequence <SEQ ID 33>:

```
  1  ATGAAATACT TGATCCGCAC CGCCTTACTC GCAGTCGCAG CCGCCGGCAT
 51  CTACGCCTGC CAACCGCAAT CCGAAGCCGC AGTGCAAGTC AAGGCTGAAA
101  ACAGCCTGAC CGCTATGCGC TTAGCCGTCG CCGACAAACA GGCAGAGATT
151  GACGGGTTGA ACGCCCAAAT CGACGCCGAA ATCAGACAAC GCGAAGCCGA
201  AGAATTGAAA GACTACCGAT GGATACACGG CGACGCGGAA GTGCCGGAGC
251  TGGAAAAATG A
```

This corresponds to the amino acid sequence <SEQ ID 34; ORF52-1>:

```
 1  MKYLIRTALL AVAAAGIYAC QPQSEAAVQV KAENSLTAMR LAVADKQAEI
51  DGLNAQIDAE IRQREAEELK DYRWIHGDAE VPELEK*
```

Computer analysis of this amino acid sequence predicts a prokaryotic membrane lipoprotein lipid attachment site (underlined).

ORF52-1 (7kDa) was cloned in the pGex vectors and expressed in *E.coli*, as described above. The products of protein expression and purification were analyzed by SDS-PAGE. Figure 4A shows the results of affinity purification of the GST-fusion. Figure 4B shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF52-1.

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 8

The following DNA sequence was identified in *N.meningitidis* <SEQ ID 35>

```
  1  ATGGTTATCG GAATATTACT CGCATCAAGC AAGCATGCTC TTGTCATTAC
 51  TCTATTGTTA AATCCCGTCT TCCATGCATC CAGTTGCGTA TCGCGTTaGG
101  CAATACGGAA TAAAAtCTGC TGTTCTGCTT TGGCTAAATT TGCCAAATTG
151  TTTATTGTTT CTTTAGGaGC AGCTTGCTTA GCCGCCTTCG CTTTCGACAA
201  CGCCCCCACA GGCGCTTCCC AAGCgTTGCC TACCGTTACC GCACCCGTGG
251  CGATTCCCGC GCCCGCTTCG GCAGCCTGA
```

This corresponds to the amino acid sequence <SEQ ID 36; ORF56>:

```
  1  MVIGILLASS KHALVITLLL NPVFHASSCV SRXAIRNKIC CSALAKFAKL
 51  FIVSLGAACL AAFAFDNAPT GASQALPTVT APVAIPAPAS AA*
```

Further work revealed the complete nucleotide sequence <SEQ ID 37>:

```
  1  ATGGCTTGTA CAGGTTTGAT GGTTTTTCCG TTAATGGTTA TCGGAATATT
 51  ACTTGCATCA AGCAAGCCTG CTCCTTTCCT TACTCTATTG TTAAATCCCG
101  TCTTCCATGC ATCCAGTTGC GTATCGCGTT GGGCAATACG GAATAAAATC
151  TGCTGTTCTG CTTTGGCTAA ATTTGCCAAA TTGTTTATTG TTTCTTTAGG
201  AGCAGCTTGC TTAGCCGCCT TCGCTTTCGA CAACGCCCCC ACAGGCGCTT
251  CCCAAGCGTT GCCTACCGTT ACCGCACCCG TGGCGATTCC CGCGCCCGCT
301  TCGGCAGCCT GA
```

This corresponds to the amino acid sequence <SEQ ID 38; ORF56-1>:

```
  1  MACTGLMVFP LMVIGILLAS SKPAPFLTLL LNPVFHASSC VSRWAIRNKI
 51  CCSALAKFAK LFIVSLGAAC LAAFAFDNAP TGASQALPTV TAPVAIPAPA
101  SAA*
```

Computer analysis of this amino acid sequence predicts a leader peptide (underlined) and suggests that ORF56 might be a membrane or periplasmic protein.

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 9

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 39>

```
  1  ATGTTCAGTA TTTTAAATGT GTTTCTTCAT TGTATTCTGG CTTGTGTAGT
 51  CTCTGGTGAG ACGCCTACTA TATTTGGTAT CCTTGCTCTT TTTTACTTAT
101  TGTATCTTTC TTATCTTGCT GTTTTTAAGA TTTTCTTTTC TTTTTTCTTA
151  GACAGAGTTT CACTCCGGTC TCCCAGGCTG GAGTGCAAAT GGCATGACCC
201  TTTGGCTCAC TGGCTCACGG CCACTTCTGC TATTCTGCCG CCTCAGCCTC
251  CAGGG...
```

This corresponds to the amino acid sequence <SEQ ID 40; ORF63>:

```
  1  MFSILNVFLH CILACVVSGE TPTIFGILAL FYLLYLSYLA VFKIFFSFFL
 51  DRVSLRSPRL ECKWHDPLAH WLTATSAILP PQPPG...
```

Computer analysis of this amino acid sequence predicts a transmembrane region.

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 10

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 41>

```
  1  ..GTGCGGACGT GGTTGGTTTT TTGGTTGCAG CGTTTGAAAT ACCCGTTGTT
 51    GCTTTGGATT GCGGATATGT TGCTGTACCG GTTGTTGGGC GGCGCGGAAA
101    TCGAATGCGG CCGTTGCCCT GTGCCGCCGA TGACGGATTG GCAGCATTTT
151    TTGCCGGCGA TGGGAACGGT GTCGGCTTGG GTGGCGGTGA TTTGGGCATA
201    CCTGATGATT GAAAGTGAAA AAAACGGAAG ATATTGA
```

This corresponds to the amino acid sequence <SEQ ID 42; ORF69>:

```
  1  ..VRTWLVFWLQ RLKYPLLLWI ADMLLYRLLG GAEIECGRCP VPPMTDWQHF
 51    LPAMGTVSAW VAVIWAYLMI ESEKNGRY*
```

Computer analysis of this amino acid sequence predicts a transmembrane region.

A corresponding ORF from strain A of *N.meningitidis* was also identified:

<u>Homology with a predicted ORF from *N.meningitidis* (strain A)</u>

ORF69 shows 96.2% identity over a 78aa overlap with an ORF (ORF69a) from strain A of *N. meningitidis*:

```
                     10        20        30        40        50        60
orf69.pep    VRTWLVFWLQRLKYPLLLWIADMLLYRLLGGAEIECGRCPVPPMTDWQHFLPAMGTVSAW
             |||||||||||||||||| |||||||||||||||||||||||||||||||||:||||:||
orf69a       VRTWLVFWLQRLKYPLLLCIADMLLYRLLGGAEIECGRCPVPPMTDWQHFLPTHGTVAAW
                     10        20        30        40        50        60

                     70       79
orf69.pep    VAVIWAYLMIFSEKNGRYX
```

```
              ||||||||||||||||||
orf69a        VAVIWAYLMIESEKNGRYX
                       70
```

The ORF69a nucleotide sequence <SEQ ID 43> is:

```
  1  GTGCGGACGT GGTTGGTTTT TTGGTTGCAG CGTTTGAAAT ACCCGTTGTT
 51  GCTTTGTATT GCGGATATGC TGCTGTACCG GTTGTTGGGC GGCGCGGAAA
101  TCGAATGCGG CCGTTGCCCT GTACCGCCGA TGACGGATTG GCAGCATTTT
151  TTGCCGACGA TGGGAACGGT GGCGGCTTGG GTGGCGGTGA TTTGGGCATA
201  CCTGATGATT GAAAGTGAAA AAAACGGAAG ATATTGA
```

This encodes a protein having amino acid sequence <SEQ ID 44>:

```
  1  VRTWLVFWLQ RLKYPLLLCI ADMLLYRLLG GAEIECGRCP VPPMTDWQHF
 51  LPTMGTVAAW VAVIWAYLMI ESEKNGRY*
```

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 11

The following DNA sequence was identified in *N.meningitidis* <SEQ ID 45>

```
  1  ATGTTTCAAA ATTTTGATTT GGGCGTGTTC CTGCTTGCCG TCCTCCCCGT
 51  GCTGCCCTCC ATTACCGTCT CGCACGTGGC GCGCGGCTAT ACGGCGCGCT
101  ACTGGGGAGA CAACACTGCC GAACAATACG GCAGGCTGAC ACTGAACCCC
151  CTGCCCCATA TCGATTTGGT CGGCACAATC ATCgTACCGC TGCTTACTTT
201  GATGTTCACG CCCTTCCTGT TCGGCTGGGC GCGTCCGATT CCTATCGATT
251  CGCGCAACTT CCGCAACCCG cGCCTTGCCT GGCGTTGCGT TGCCGCGTCC
301  GGCCCGCTGT CGAATCTAGC GATGGCTGTw CTGTGGGGCG TGGTTTTGGT
351  GCTGACTCCG TATGTCGGCG GGGCGTATCA GATGCCGTTG GCTCAAATGG
401  CAAACTACGG TATTCTGATC AATGCGATTC TGTTCGCGCT CAACATCATC
451  CCCATCCTGC CTTGGGACGG CGGCATTTTC ATCGACACCT TCCTGTCGGC
501  GAAATATTCG CAAGCGTTCC GCAAAATCGA ACCTTATGGG ACGTGGATTA
551  TCCTACTGCT GATGCTGACC sGGGTTTTGG GTGCGTTTAT wGCACCGATT
601  aTGCGGmTGc GTGATTGCrT TTGTGCAGAT GTwCGTCTGA CTGGCTTTCA
651  GACGGCATAA
```

This corresponds to the amino acid sequence <SEQ ID 46; ORF77>:

```
  1  MFQNFDLGVF LLAVLPVLPS ITVSHVARGY TARYWGDNTA EQYGRLTLNP
 51  LPHIDLVGTI IVPLLTLMFT PFLFGWARPI PIDSRNFRNP RLAWRCVAAS
101  GPLSNLAMAV LWGVVLVLTP YVGGAYQMPL AQMANYGILI NAILFALNII
151  PILPWDGGIF IDTFLSAKYS QAFRKIEPYG TWIILLLMLT XVLGAFIAPI
201  XRXRDCXCAD VRLTGFQTA*
```

Further work revealed the complete nucleotide sequence <SEQ ID 47>:

```
  1  ATGTTTCAAA ATTTTGATTT GGGCGTGTTT CTGCTTGCCG TCCTGCCCGT
 51  GCTGCTCTCC ATTACCGTCA GGGAGGTGGC GCGCGGCTAT ACGGCGCGCT
101  ACTGGGGAGA CAACACTGCC GAACAATACG GCAGGCTGAC ACTGAACCCC
151  CTGCCCCATA TCGATTTGGT CGGCACAATC ATCGTACCGC TGCTTACTTT
201  GATGTTCACG CCCTTCCTGT TCGGCTGGGC GCGTCCGATT CCTATCGATT
251  CGCGCAACTT CCGCAACCCG CGCCTTGCCT GGCGTTGCGT TGCCGCGTCC
301  GGCCCGCTGT CGAATCTAGC GATGGCTGTT CTGTGGGGCG TGGTTTTGGT
351  GCTGACTCCG TATGTCGGCG GGGCGTATCA GATGCCGTTG GCTCAAATGG
401  CAAACTACGG TATTCTGATC AATGCGATTC TGTTCGCGCT CAACATCATC
```



```
451  CCCATCCTGC CTTGGGACGG CGGCATTTTC ATCGACACCT TCCTGTCGGC
501  GAAATATTCG CAAGCGTTCC GCAAAATCGA ACCTTATGGG ACGTGGATTA
551  TCCTACTGCT GATGCTGACC GGGGTTTTGG GTGCGTTTAT TGCACCGATT
601  GTGCGGCTGG TGATTGCGTT TGTGCAGATG TTCGTCTGA
```

This corresponds to the amino acid sequence <SEQ ID 48; ORF77-1>:

```
  1  MFQNFDLGVF LLAVLPVLLS ITVREVARGY TARYWGDNTA EQYGRLTLNP
 51  LPHIDLVGTI IVPLLTLMFT PFLFGWARPI PIDSRNFRNP RLAWRCVAAS
101  GPLSNLAMAV LWGVVLVLTP YVGGAYQMPL AQMANYGILI NAILFALNII
151  PILPWDGGIF IDTFLSAKYS QAFRKIEPYG TWIILLLMLT GVLGAFIAPI
201  VRLVIAFVQM FV*
```

Computer analysis of this amino acid sequence reveals a putative leader sequence and several transmembrane domains.

A corresponding ORF from strain A of *N.meningitidis* was also identified:

<u>Homology with a predicted ORF from *N.meningitidis* (strain A)</u>

ORF77 shows 96.5% identity over a 173aa overlap with an ORF (ORF77a) from strain A of *N. meningitidis:*

```
                   10        20        30        40        50        60
orf77.pep   MFQNFDLGVFLLAVLPVLPSITVSHVARGYTARYWGDNTAEQYGRLTLNPLPHIDLVGTI
                                        ||||||||||||||||||||||||||||||
orf77a                                  RGYTARYWGDNTAEQYGRLTLNPLPHIDLVGTI
                                                10        20        30

                   70        80        90       100       110       120
orf77.pep   IVPLLTLMFTPFLFGWARPIPIDSRNFRNPRLAWRCVAASGPLSNLAMAVLWGVVLVLTP
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf77a      IVPLLTLMFTPFLFGWARPIPIDSRNFRNPRLAWRCVAASGPLSNLAMAVLWGVVLVLTP
                   40        50        60        70        80        90

                  130       140       150       160       170       180
orf77.pep   YVGGAYQMPLAQMANYGILINAILFALNIIPILPWDGGIFIDTFLSAKYSQAFRKIEPYG
            ||||||||||||||| ||||||| ||||||||||||||||||||||||| ||||||||||
orf77a      YVGGAYQMPLAQMANXILINAILXALNIIPILPWDGGIFIDTFLSAKXSQAFRKIEPYG
                  100       110       120       130       140       150

                  190       200       210       220
orf77.pep   TWIILLLMLTXVLGAFIAPIXRXRDCXCADVRLTGFQTAX
            |||| ||||| |||| ||||
orf77a      TWIIXLLMLTGVLGAXIAPIVQLVIAFVQMFVX
                  160       170       180
```

ORF77-1 and ORF77a show 96.8% identity in 185 aa overlap:

```
                   10        20        30        40        50        60
orf77-1.pep MFQNFDLGVFLLAVLPVLLSITVREVARGYTARYWGDNTAEQYGRLTLNPLPHIDLVGTI
                                        ||||||||||||||||||||||||||||||
orf77a                                  RGYTARYWGDNTAEQYGRLTLNPLPHIDLVGTI
                                                10        20        30

                   70        80        90       100       110       120
orf77-1.pep IVPLLTLMFTPFLFGWARPIPIDSRNFRNPRLAWRCVAASGPLSNLAMAVLWGVVLVLTP
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
```

```
orf77a         IVPLLTLMFTPFLFGWARPIPIDSRNFRNPRLAWRCVAASGPLSNLAMAVLWGVVLVLTP
                       40        50        60        70        80        90

                      130       140       150       160       170       180
orf77-1.pep    YVGGAYQMPLAQMANYGILINAILFALNIIPILPWDGGIFIDTFLSAKYSQAFRKIEPYG
               ||||||||||||||||| |||||||| |||||||||||||||||||||||| |||||||||||
orf77a         YVGGAYQMPLAQMANYXILINAILXALNIIPILPWDGGIFIDTFLSAKXSQAFRKIEPYG
                      100       110       120       130       140       150

                      190       200       210
orf77-1.pep    TWIILLLMLTGVLGAFIAPIVRLVIAFVQMFVX
               |||| |||||||||| |||||:|||||||||||
orf77a         TWIIXLLMLTGVLGAXIAPIVQLVIAFVQMFVX
                      160       170       180
```

A partial ORF77a nucleotide sequence <SEQ ID 49> was identified:

```
  1  ..CGCGGCTATA CAGCGCGCTA CTGGGGTGAC AACACTGCCG AACAATACGG
 51    CAGGCTGACA CTGAACCCCC TGCCCCATAT CGATTTGGTC GGCACAATCA
101    TCGTACCGCT GCTTACTTTG ATGTTTACGC CCTTCCTGTT CGGCTGGGCG
151    CGTCCGATTC CTATCGATTC GCGCAACTTC CGCAACCCGC GCCTTGCCTG
201    GCGTTGCGTT GCCGCGTCCG GCCCGCTGTC GAATCTGGCG ATGGCTGTTC
251    TGTGGGGCGT GGTTTTGGTG CTGACTCCGT ATGTCGGTGG GGCGTATCAG
301    ATGCCGTTGG CNCAAATGGC AAACTACNNN ATTCTGATCA ATGCGATTCT
351    GTNCGCGCTC AACATCATCC CCATCCTGCC TTGGGACGGC GGCATTTTCA
401    TCGACACCTT CCTGTCGGCN AAATANTCGC AAGCGTTCCG CAAAATCGAA
451    CCTTATGGGA CGTGGATTAT CCNGCTGCTT ATGCTGACCG GGGTTTTGGG
501    TGCGTNTATT GCACCGATTG TGCAGCTGGT GATTGCGTTT GTGCAGATGT
551    TCGTCTGA
```

This encodes a protein having amino acid sequence <SEQ ID 50>:

```
  1  ..RGYTARYWGD NTAEQYGRLT LNPLPHIDLV GTIIVPLLTL MFTPFLFGWA
 51    RPIPIDSRNF RNPRLAWRCV AASGPLSNLA MAVLWGVVLV LTPYVGGAYQ
101    MPLAQMANYX ILINAILXAL NIIPILPWDG GIFIDTFLSA KXSQAFRKIE
151    PYGTWIIXLL MLTGVLGAXI APIVQLVIAF VQMFV*
```

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 12

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 51>

```
  1  ATGAACCTGA TTTCACGTTA CATCATCCGT CAAATGGCGG TTATGGCGGT
 51  TTACGCGCTC CTTGCCTTCC TCGCTTTGTA CAGCTTTTTT GAAATCCTGT
101  ACGAAACCGG CAACCTCGGC AAAGGCAGTT ACGGCATATG GGAAATGCTG
151  GGCTACACCG CCCTCAAAAT GCCCGCCCGC GCCTACGAAC TGATTCCCCT
201  CGCCGTCCTT ATCGGCGGAC TGGTCTCCCT CAGCCAGCTT GCCGCCGGCA
251  GCGAACTGAC CGTCATCAAA GCCAGCGGCA TGAGCACCAA AAAGCTGCTG
301  TTGATTCTGT CGCAGTTCGG TTTTATTTTT GCTATTGCCA CCGTCGCGCT
351  CGGCGAATGG GTTGCGCCCA CACTGAGCCA AAAAGCCGAA AACATCAAAG
401  CCGCCGCCAT CAACGGCAAA ATCAGCACCG GCAATACCGG CCTTTGGCTG
451  AAAGAAAAAA ACAGCGTGAT CAATGTGCGC GAAATGTTGC CCGACCAT..
```

This corresponds to the amino acid sequence <SEQ ID 52; ORF112>:

```
  1  MNLISRYIIR QHAVMAVYAL LAFLALYSFF EILYETGNLG KGSYGIWEML
```



```
 51  GYTALKMPAR AYELIPLAVL IGGLVSLSQL AAGSELTVIK ASGMSTKKLL
101  LILSQFGFIF AIATVALGEW VAPTLSQKAE NIKAAAINGK ISTGNTGLWL
151  KEKNSVINVR EMLPDH...
```

Further work revealed further partial nucleotide sequence <SEQ ID 53>:

```
  1  ATGAACCTGA TTTCACGTTA CATCATCCGT CAAATGGCGG TTATGGCGGT
 51  TTACGCGCTC CTTGCCTTCC TCGCTTTGTA CAGCTTTTTT GAAATCCTGT
101  ACGAAACCGG CAACCTCGGC AAAGGCAGTT ACGGCATATG GGAAATGCTG
151  gGCTACACCG CCCTCAAAAT GCCCGCCCGC GCCTACGAAC TGATTCCCCT
201  CGCCGTCCTT ATCGGCGGAC TGGTCTCCCT CAGCCAGCTT GCCGCCGGCA
251  GCGAACTGAC CGTCATCAAA GCCAGCGGCA TGAGCACCAA AAAGCTGCTG
301  TTGATTCTGT CGCAGTTCGG TTTTATTTTT GCTATTGCCA CCGTCGCGCT
351  CGGCGAATGG GTTGCGCCCA CACTGAGCCA AAAAGCCGAA AACATCAAAG
401  CCGCCGCCAT CAACGGCAAA ATCAGCACCG GCAATACCGG CCTTTGGCTG
451  AAAGAAAAAA ACAGCrTkAT CAATGTGCGC GAAATGTTGC CCGACCATAC
501  GCTTTTGGGC ATCAAAATTT GGGCGCGCAA CGATAAAAAC GAATTGGCAG
551  AGGCAGTGGA AGCCGATTCC GCCGTTTTGA ACAGCGACGG CAGTTGGCAG
601  TTGAAAAACA TCCGCCGCAG CACGCTTGGC GAAGACAAAG TCGAGGTCTC
651  TATTGCGGCT GAAGAAAACT GGCCGATTTC CGTCAAACGC AACCTGATGG
701  ACGTATTGCT CGTCAAACCC GACCAAATGT CCGTCGGCGA ACTGACCACC
751  TACATCCGCC ACCTCCAAAA CAACAGCCAA AACACCCGAA TCTACGCCAT
801  CGCATGGTGG CGCAAATTGG TTTACCCCGC CGCAGCCTGG GTGATGGCGC
851  TCGTCGCCTT TGCCTTTACC CCGCAAACCA CCCGCCACGG CAATATGGGC
901  TTAAAACTCT TCGGCGGCAT CTGTsTCGGA TTGCTGTTCC ACCTTGCCGG
951  ACGGCTCTTT GGGTTTACCA GCCAACTCGG...
```

This corresponds to the amino acid sequence <SEQ ID 54; ORF112-1>:

```
  1  MNLISRYIIR QHAVMAVYAL LAFLALYSFF EILYETGNLG KGSYGIWEML
 51  GYTALKMPAR AYELIPLAVL IGGLVSLSQL AAGSELTVIK ASGMSTKKLL
101  LILSQFGFIF AIATVALGEW VAPTLSQKAE NIKAAAINGK ISTGNTGLWL
151  KEKNSXINVR EMLPDHTLLG IKIWARNDKN ELAEAVEADS AVLNSDGSWQ
201  LKNIRRSTLG EDKVEVSIAA-EENWPISVKR NLMDVLLVKP DQMSVGELTT
251  YIRHLQNNSQ NTRIYAIAWW RKLVYPAAAW VMALVAFAFT PQTTRHGNMG
301  LKLFGGICXG LLFHLAGRLF GFTSQL...
```

Computer analysis of this amino acid sequence predicts two transmembrane domains.

A corresponding ORF from strain A of *N.meningitidis* was also identified:

<u>Homology with a predicted ORF from *N.meningitidis* (strain A)</u>

ORF112 shows 96.4% identity over a 166aa overlap with an ORF (ORF112a) from strain A of *N. meningitidis*:

```
                      10        20        30        40        50        60
orf112.pep     MNLISRYIIRQHAVMAVYALLAFLALYSFFEILYETGNLGKGSYGIWEMLGYTALKMPAR
               |||||||||||||||||||||||||||||||||||||||||||||||| ||||||| ||
orf112a        MNLISRYIIRQHAVMAVYALLAFLALYSFFEILYETGNLGKGSYGIWEMXGYTALKMXAR
                      10        20        30        40        50        60

                      70        80        90       100       110       120
orf112.pep     AYELIPLAVLIGGLVSLSQLAAGSELTVIKASGMSTKKLLLILSQFGFIFAIATVALGEW
               ||||:|||||||||||| ||||||||:|||||||||||||||||||||||||||||||||
orf112a        AYELMPLAVLIGGLVSXSQLAAGSELXVIKASGMSTKKLLLILSQFGFIFAIATVALGEW
                      70        80        90       100       110       120

                     130       140       150       160
```

```
orf112.pep  VAPTLSQKAENIKAAAINGKISTGNTGLWLKEKNSVINVREMLPDH
            ||||||||||||||||||||||||||||||||||||:|||||||||
orf112a     VAPTLSQKAENIKAAAINGKISTGNTGLWLKEKNSIINVREMLPDHTLLGIKIWARNDKN
                    130       140       150       160       170       180

orf112a     ELAEAVEADSAVLNSDGSWQLKNIRRSTLGEDKVEVSIAAEEXWPISVKRNLMDVLLVKP
                    190       200       210       220       230       240
```

A partial ORF112a nucleotide sequence <SEQ ID 55> was identified:

```
   1  ATGAACCTGA TTTCACGTTA CATCATCCGT CAAATGGCGG TTATGGCGGT
  51  TTACGCGCTC CTTGCCTTCC TCGCTTTGTA CAGCTTTTTT GAAATCCTGT
 101  ACGAAACCGG CAACCTCGGC AAAGGCAGTT ACGGCATATG GGAAATGNTG
 151  GGNTACACCG CCCTCAAAAT GNCCGCCCGC GCCTACGAAC TGATGCCCCT
 201  CGCCGTCCTT ATCGGCGGAC TGGTCTCTNT CAGCCAGCTT GCCGCCGGCA
 251  GCGAACTGAN CGTCATCAAA GCCAGCGGCA TGAGCACCAA AAAGCTGCTG
 301  TTGATTCTGT CGCAGTTCGG TTTTATTTTT GCTATTGCCA CCGTCGCGCT
 351  CGGCGAATGG GTTGCGCCCA CACTGAGCCA AAAAGCCGAA AACATCAAAG
 401  CCGCGGCCAT CAACGGCAAA ATCAGTACCG GCAATACCGG CCTTTGGCTG
 451  AAAGAAAAAA ACAGCATTAT CAATGTGCGC GAAATGTTGC CCGACCATAC
 501  CCTGCTGGGC ATTAAAATCT GGGCCCGCAA CGATAAAAAC GAACTGGCAG
 551  AGGCAGTGGA AGCCGATTCC GCCGTTTTGA ACAGCGACGG CAGTTGGCAG
 601  TTGAAAAACA TCCGCCGCAG CACGCTTGGC GAAGACAAAG TCGAGGTCTC
 651  TATTGCGGCT GAAGAAAANT GGCCGATTTC CGTCAAACGC AACCTGATGG
 701  ACGTATTGCT CGTCAAACCC GACCAAATGT CCGTCGGCGA ACTGACCACC
 751  TACATCCGCC ACCTCCAAAN NNACAGCCAA AACACCCGAA TCTACGCCAT
 801  CGCATGGTGG CGCAAATTGG TTTACCCCGC CGCAGCCTGG GTGATGGCGC
 851  TCGTCGCCTT TGCCTTTACC CCGCAAACCA CCCGCCACGG CAATATGGGC
 901  TTAAAANTCT TCGGCGGCAT CTGTCTCGGA TTGCTGTTCC ACCTTGCCGG
 951  NCGGCTCTTC NGGTTTACCA GCCAACTCTA CGGCATCCCG CCCTTCCTCG
1001  NCGGCGCACT ACCTACCATA GCCTTCGCCT TGCTCGCCGT TTGGCTGATA
1051  CGCAAACAGG AAAAACGCTA A
```

This encodes a protein having amino acid sequence <SEQ ID 56>:

```
   1  MNLISRYIIR QMAVMAVYAL LAFLALYSFF EILYETGNLG KGSYGIWEMX
  51  GYTALKMXAR AYELMPLAVL IGGLVSXSQL AAGSELXVIK ASGMSTKKLL
 101  LILSQFGFIF AIATVALGEW VAPTLSQKAE NIKAAAINGK ISTGNTGLWL
 151  KEKNSIINVR EMLPDHTLLG IKIWARNDKN ELAEAVEADS AVLNSDGSWQ
 201  LKNIRRSTLG EDKVEVSIAA EEXWPISVKR NLMDVLLVKP DQMSVGELTT
 251  YIRHLQXXSQ NTRIYAIAWW RKLVYPAAAW VMALVAFAFT PQTTRHGNMG
 301  LKXFGGICLG LLFHLAGRLF XFTSQLYGIP PFLXGALPTI AFALLAVWLI
 351  RKQEKR*
```

ORF112a and ORF112-1 show 96.3% identity in 326 aa overlap:

```
orf112a.pep  MNLISRYIIRQMAVMAVYALLAFLALYSFFEILYETGNLGKGSYGIWEMXGYTALKMXAR
             ||||||||||||||||||||||||||||||||||||||||||||||||| |||||| ||
orf112-1     MNLISRYIIRQMAVMAVYALLAFLALYSFFEILYETGNLGKGSYGIWENLGYTALKMPAR

orf112a.pep  AYELMPLAVLIGGLVSXSQLAAGSELXVIKASGMSTKKLLLILSQFGFIFAIATVALGEW
             ||||:|||||||||||| |||||||||:||||||||||||||||||||||||||||||||
orf112-1     AYELIPLAVLIGGLVSLSQLAAGSELTVIKASGMSTKKLLLILSQFGFIFAIATVALGEW

orf112a.pep  VAPTLSQKAENIKAAAINGKISTGNTGLWLKEKNSIINVREMLPDHTLLGIKIWARNDKN
             ||||||||||||||||||||||||||||||||||| ||||||||||||||||||||||||
orf112-1     VAPTLSQKAENIKAAAINGKISTGNTGLWLKEKNSXINVREMLPDHTLLGIKIWARNDKN

orf112a.pep  ELAEAVEADSAVLNSDGSWQLKNIRRSTLGEDKVEVSIAAEEXWPISVKRNLMDVLLVKP
             |||||||||||||||||||||||||||||||||||||||||| |||||||||||||||||
orf112-1     ELAEAVEADSAVLNSDGSWQLKNIRRSTLGEDKVEVSIAAEENWPISVKRNLMDVLLVKP

orf112a.pep  DQMSVGELTTYIRHLQXXSQNTRIYAIAWWRKLVYPAAAWVMALVAFAFTPQTTRHGNMG
```



```
             ||||||||||||| |||||||||||||||||||||||||||||||||||||||||
orf112-1     DQMSVGELTTYIRHLQNNSQNTRIYAIAWWRKLVYPAAAWVMALVAFAFTPQTTRHGNMG

orf112a.pep  LKXFGGICLGLLFHLAGRLFXFTSQLYGIPPFLXGALPTIAFALLAVWLIRKQEKRX
             || ||||| |||||||||| |||||
orf112-1     LKLFGGICXGLLFHLAGRLFGFTSQL
```

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 13

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 57>

```
   1  ..GCAGTAGCCG AAACTGCCAA CAGCCAGGGC AAAGGTAAAC AGGCAGGCAG
  51    TTCGGTTTCT GTTTCACTGA AAACTTCAGG CGACCTTTGC GGCAAACTCA
 101    AAACCACCCT TAAAACTTTG GTCTGCTCTT TGGTTTCCCT GAGTATGGTA
 151    TTGCCTGCCC ATGCCCAAAT TACCACCGAC AAATCAGCAC CTAAAAACCA
 201    GCAGGTCGTT ATCCTTAAAA CCAACACTGG TGCCCCCTTG GTGAATATCC
 251    AAACTCCGAA TGGACGCGGA TTGAGCCACA ACCGCTA.TA CGCATTTGAT
 301    GTTGACAACA AAGGGGCAGT GTTAAACAAC GACCGTAACA ATAATCCGTT
 351    TGTGGTCAAA GGCAGTGCGC AATTGATTTT GAACGAGGTA CGCGGTACGG
 401    CTAGCAAACT CAACGGCATC GTTACCGTAG GCGGTCAAAA GGCCGACGTG
 451    ATTATTGCCA ACCCCAACGG CATTACCGTT AATGGCGGCG GCTTTAAAAA
 501    TGTCGGTCGG GGCATCTTAA CTACCGGTGC GCCCCAAATC GGCAAAGACG
 551    GTGCACTGAC AGGATTTGAT GTGCGTCAAG GCACATTGgA CCGTAGrAGC
 601    AGCAGGTTGG AATGATAAAG GCGGAGCmrm yTACACCGGG GTACTTGCTC
 651    GTGCAGTTGC TTTGCAGGGG AAATTwmmGG GTAAA.AACT GGCGGTTTCT
 701    ACCGGTCCTC AGAAAGTAGA TTACGCCAGC GGCGAAATCA GTGCAGGTAC
 751    GGCAGCGGGT ACGAAACCGA CTATTGCCCT TGATACTGCC GCACTGGGCG
 801    GTATGTACGC CGACAGCATC ACACTGATTG CCAATGAAAA AGGCGTAGGC
 851    GTCTAA
```

This corresponds to the amino acid sequence <SEQ ID 58; ORF114>:

```
   1  ..AVAETANSQG KGKQAGSSVS VSLKTSGDLC GKLKTTLKTL VCSLVSLSMV
  51    LPAHAQITTD KSAPKNQQVV ILKTNTGAPL VNIQTPNGRG LSHNRXYAFD
 101    VDNKGAVLNN DRNNNPFVVK GSAQLILNEV RGTASKLNGI VTVGGQKADV
 151    IIANPNGITV NGGGFKNVGR GILTTGAPQI GKDGALTGFD VVKAHWTVXA
 201    AGWNDKGGAX YTGVLARAVA LQGKXXGKXL AVSTGPQKVD YASGEISAGT
 251    AAGTKPTIAL DTAALGGMYA DSITLIANEK GVGV*
```

Further work revealed the complete nucleotide sequence <SEQ ID 59>:

```
   1  ATGAATAAAG GTTTACATCG CATTATCTTT AGTAAAAAGC ACAGCACCAT
  51  GGTTGCAGTA GCCGAAACTG CCAACAGCCA GGGCAAAGGT AAACAGGCAG
 101  GCAGTTCGGT TTCTGTTTCA CTGAAAACTT CAGGCGACCT TTGCGGCAAA
 151  CTCAAAACCA CCCTTAAAAC TTTGGTCTGC TCTTTGGTTT CCCTGAGTAT
 201  GGTATTGCCT GCCCATGCCC AAATTACCAC CGACAAATCA GCACCTAAAA
 251  ACCAGCAGGT CGTTATCCTT AAAACCAACA CTGGTGCCCC CTTGGTGAAT
 301  ATCCAAACTC CGAATGGACG CGGATTGAGC CACAACCGCT ATACGCAGTT
 351  TGATGTTGAC AACAAAGGGG CAGTGTTAAA CAACGACCGT AACAATAATC
 401  CGTTTGTGGT CAAAGGCAGT GCGCAATTGA TTTTGAACGA GGTACGCGGT
 451  ACGGCTAGCA AACTCAACGG CATCGTTACC GTAGGCGGTC AAAAGGCCGA
 501  CGTGATTATT GCCAACCCCA ACGGCATTAC CGTTAATGGC GGCGGCTTTA
 551  AAAATGTCGG TCGGGGCATC TTAACTACCG GTGCGCCCCA AATCGGCAAA
 601  GACGGTGCAC TGACAGGATT TGATGTGCGT CAAGGCACAT TGACCGTAGG
 651  AGCAGCAGGT TGGAATGATA AAGGCGGAGC CGACTACACC GGGGTACTTG
 701  CTCGTGCAGT TGCTTTGCAG GGGAAATTAC AGGGTAAAAA CCTGGCGGTT
```

```
751   TCTACCGGTC CTCAGAAAGT AGATTACGCC AGCGGCGAAA TCAGTGCAGG
801   TACGGCAGCG GGTACGAAAC CGACTATTGC CCTTGATACT GCCGCACTGG
851   GCGGTATGTA CGCCGACAGC ATCACACTGA TTGCCAATGA AAAAGGCGTA
901   GGCGTCAAAA ATGCCGGCAC ACTCGAAGCG GCCAAGCAAT TGATTGTGAC
951   TTCGTCAGGC CGCATTGAAA ACAGCGGCCG CATCGCCACC ACTGCCGACG
1001  GCACCGAAGC TTCACCGACT TATCTCTCCA TCGAAACCAC CGAAAAAGGA
1051  GCGGCAGGCA CATTTATCTC CAATGGTGGT CGGATCGAGA GCAAAGGCTT
1101  ATTGGTTATT GAGACGGGAG AAGATATCAG CTTGCGTAAC GGAGCCGTGG
1151  TGCAGAATAA CGGCAGTCGC CCAGCTACCA CGGTATTAAA TGCTGGTCAT
1201  AATTTGGTGA TTGAGAGCAA AACTAATGTG AACAATGCCA AAGGCCCGGC
1251  TACTCTGTCG GCCGACGGCC GTACCGTCAT CAAGGAGGCC AGTATTCAGA
1301  CTGGCACTAC CGTATACAGT TCCAGCAAAG GCAACGCCGA ATTAGGCAAT
1351  AACACACGCA TTACCGGGGC AGATGTTACC GTATTATCCA ACGGCACCAT
1401  CAGCAGTTCC GCCGTAATAG ATGCCAAAGA CACCGCACAC ATCGAAGCAG
1451  GCAAACCGCT TTCTTTGGAA GCTTCAACAG TTACCTCCGA TATCCGCTTA
1501  AACGGAGGCA GTATCAAGGG CGGCAAGCAG CTTGCTTTAC TGGCAGACGA
1551  TAACATTACT GCCAAAACTA CCAATCTGAA TACTCCCGGC AATCTGTATG
1601  TTCATACAGG TAAAGATCTG AATTTGAATG TTGATAAAGA TTTGTCTGCC
1651  GCCAGCATCC ATTTGAAATC GGATAACGCT GCCCATATTA CCGGCACCAG
1701  TAAAACCCTC ACTGCCTCAA AAGACATGGG TGTGGAGGCA GGCTCGCTGA
1751  ATGTTACCAA TACCAATCTG CGTACCAACT CGGGTAATCT GCACATTCAG
1801  GCAGCCAAAG GCAATATTCA GCTTCGCAAT ACCAAGCTGA ACGCAGCCAA
1851  GGCTCTCGAA ACCACCGCAT TGCAGGGCAA TATCGTTTCA GACGGCCTTC
1901  ATGCTGTTTC TGCAGACGGT CATGTATCCT TATTGGCCAA CGGTAATGCC
1951  GACTTTACCG GTCACAATAC CCTGACAGCC AAGGCCGATG TCAATGCAGG
2001  ATCGGTTGGT AAAGGCCGTC TGAAAGCAGA CAATACCAAT ATCACTTCAT
2051  CTTCAGGAGA TATTACGTTG GTTGCCGGCA ACGGTATTCA GCTTGGTGAC
2101  GGAAAACAAC GCAATTCAAT CAACGGAAAA CACATCAGCA TCAAAAACAA
2151  CGGTGGTAAT GCCGACTTAA AAAACCTTAA CGTCCATGCC AAAAGCGGGG
2201  CATTGAACAT TCATTCCGAC CGGGCATTGA GCATAGAAAA TACCAAGCTG
2251  GAGTCTACCC ATAATACGCA TCTTAATGCA CAACACGAGC GGGTAACGCT
2301  CAACCAAGTA GATGCCTACG CACACCGTCA TCTAAGCATT ACCGGCAGCC
2351  AGATTTGGCA AAACGACAAA CTGCCTTCTG CCAACAAGCT GGTGGCTAAC
2401  GGTGTATTGG CACTCAATGC GCGCTATTCC CAAATTGCCG ACAACACCAC
2451  GCTGAGAGCG GGTGCAATCA ACCTTACTGC CGGTACCGCC CTAGTCAAGC
2501  GCGGCAACAT CAATTGGAGT ACCGTTTCGA CCAAAACTTT GGAAGATAAT
2551  GCCGAATTAA AACCATTGGC CGGACGGCTG AATATTGAAG CAGGTAGCGG
2601  CACATTAACC ATCGAACCTG CCAACCGCAT CAGTGCGCAT ACCGACCTGA
2651  GCATCAAAAC AGGCGGAAAA TTGCTGTTGT CTGCAAAAGG AGGAAATGCA
2701  GGTGCGCCTA GTGCTCAAGT TTCCTCATTG GAAGCAAAAG GCAATATCCG
2751  TCTGGTTACA GGAGAAACAG ATTTAAGAGG TTCTAAAATT ACAGCCGGTA
2801  AAAACTTGGT TGTCGCCACC ACCAAAGGCA AGTTGAATAT CGAAGCCGTA
2851  AACAACTCAT TCAGCAATTA TTTTCCTACA CAAAAAGCGG CTGAACTCAA
2901  CCAAAAATCC AAAGAATTGG AACAGCAGAT TGCGCAGTTG AAAAAAAGCT
2951  CGCCTAAAAG CAAGCTGATT CCAACCCTGC AAGAAGAACG CGACCGTCTC
3001  GCTTTCTATA TTCAAGCCAT CAACAAGGAA GTTAAAGGTA AAAAACCCAA
3051  AGGCAAAGAA TACCTGCAAG CCAAGCTTTC TGCACAAAAT ATTGACTTGA
3101  TTTCCGCACA AGGCATCGAA ATCAGCGGTT CCGATATTAC CGCTTCCAAA
3151  AAACTGAACC TTCACGCCGC AGGCGTATTG CCAAAGGCAG CAGATTCAGA
3201  GGCGGCTGCT ATTCTGATTG ACGGCATAAC CGACCAATAT GAAATTGGCA
3251  AGCCCACCTA CAAGAGTCAC TACGACAAAG CTGCTCTGAA CAAGCCTTCA
3301  CGTTTGACCG GACGTACAGG GGTAAGTATT CATGCAGCTG CGGCACTCGA
3351  TGATGCACGT ATTATTATCG GTGCATCCGA AATCAAAGCT CCCTCAGGCA
3401  GCATAGACAT CAAAGCCCAT AGTGATATTG TACTGGAGGC TGGACAAAAC
3451  GATGCCTATA CCTTCTTAAA AACCAAAGGT AAAAGCGGCA AAATCATCAG
3501  AAAAACCAAG TTTACCAGCA CCCGCGACCA CCTGATTATG CCAGCCCCCG
3551  TCGAGCTGAC CGCCAACGGC ATAACGCTTC AGGCAGGCGG CAACATCGAA
3601  GCTAATACCA CCCGCTTCAA TGCCCCTGCA GGTAAAGTTA CCCTGGTTGC
3651  GGGTGAAGAG CTGCAACTGC TGGCAGAAGA AGGCATCCAC AAGCACGAGT
3701  TGGATGTCCA AAAAAGCCGC CGCTTTATCG GCATCAAGGT AGGCAAGAGC
3751  AATTACAGTA AAAACGAACT GAACGAAACC AAATTGCCTG TCCGCGTCGT
3801  CGCCCAAACT GCAGCCACCC GTTCAGGCTG GGATACCGTG CTCGAAGGTA
3851  CCGAATTCAA AACCACGCTG GCCGGTGCGG ACATTCAGGC AGGTGTAGGC
3901  GAAAAAGCCC GTGCCGATGC GAAAATTATC CTCAAAGGCA TTGTGAACCG
```



```
3951  TATCCAGTCG GAAGAAAAAT TAGAAACCAA CTCAACCGTA TGGCAGAAAC
4001  AGGCCGGACG CGGCAGCACT ATCGAAACGC TGAAACTGCC CAGCTTCGAA
4051  AGCCCTACTC CGCCCAAACT GACCGCCCCC GGTGGCTATA TCGTCGACAT
4101  TCCGAAAGGC AATTTGAAAA CCGAAATCGA AAAGCTGGCC AAACAGCCCG
4151  AGTATGCCTA TCTGAAACAG CTCCAAGTAG CGAAAAACGT CAACTGGAAC
4201  CAGGTGCAAC TGGCTTACGA TAAATGGGAC TATAAGCAGG AAGGCTTAAC
4251  CAGAGCCGGT GCAGCGATTG TTACCATAAT CGTAACCGCA CTGACTTATG
4301  GATACGGCGC AACCGCAGCG GGCGGTGTAG CCGCTTCAGG AAGTAGTACA
4351  GCCGCAGCTG CCGGAACAGC CGCCACAACG ACAGCAGCAG CTACTACCGT
4401  TTCTACAGCG ACTGCCATGC AAACCGCTGC TTTAGCCTCC TTGTATAGCC
4451  AAGCAGCTGT ATCCATCATC AATAATAAAG GTGATGTCGG CAAAGCGTTG
4501  AAAGATCTCG GCACCAGTGA TACGGTCAAG CAGATTGTCA CTTCTGCCCT
4551  GACGGCGGGT GCATTAAATC AGATGGGCGC AGATATTGCC CAATTGAACA
4601  GCAAGGTAAG AACCGAACTG TTCAGCAGTA CGGGCAATCA AACTATTGCC
4651  AACCTTGGAG GCAGACTGGC TACCAATCTC AGTAATGCAG GTATCTCAGC
4701  TGGTATCAAT ACCGCCGTCA ACGGCGGCAG CCTGAAAGAC AACTTAGGCA
4751  ATGCCGCATT AGGAGCATTG GTTAATAGCT TCCAAGGAGA AGCCGCCAGC
4801  AAAATCAAAA CAACCTTCAG CGACGATTAT GTTGCCAAAC AGTTCGCCCA
4851  CGCTTTGGCT GGGTGTGTTA GCGGATTGGT ACAAGGAAAA TGTAAAGACG
4901  GGGCAATTGG CGCAGCAGTT GGGGAAATCG TAGCCGACTC CATGCTTGGC
4951  GGCAGAAACC CTGCTACACT CAGCGATGCG GAAAAGCATA AGGTTATCAG
5001  TTACTCGAAG ATTATTGCCG GCAGCGTGGC GGCACTCAAC GGCGGCGATG
5051  TGAATACTGC GGCGAATGCG GCTGAGGTGG CGGTAGTGAA TAATGCTTTG
5101  AATTTTGACA GTACCCCTAC CAATGCGAAA AAGCATCAAC CGCAGAAGCC
5151  CGACAAAACC GCACTGGAAA AAATTATCCA AGGTATTATG CCTGCACATG
5201  CAGCAGGTGC GATGACTAAT CCGCAGGATA AGGATGCTGC CATTTGGATA
5251  AGCAATATCC GTAATGGCAT CACAGGCCCG ATTGTGATTA CCAGCTATGG
5301  GGTTTATGCT GCAGGTTGGA CAGCTCCGCT GATCGGTACA GCGGGTAAAT
5351  TAGCTATCAG CACCTGCATG GCTAATCCTT CTGGTTGTAC TGTCATGGTC
5401  ACTCAGGCTG CCGAAGCGGG CGCGGGAATC GCCACGGGTG CGGTAACGGT
5451  AGGCAACGCT TGGGAAGCGC CTGTGGGGGC GTTGTCGAAA GCGAAGGCGG
5501  CCAAGCAGGC TATACCAACC CAGACAGTTA AAGAACTTGA TGGCTTACTA
5551  CAAGAATCAA AAAATATAGG TGCTGTAAAT ACACGAATTA ATATAGCGAA
5601  TAGTACTACT CGATATACAC CAATGAGACA AACGGGACAA CCGGTATCTG
5651  CTGGCTTTGA GCATGTTCTT GAGGGGCACT TCCATAGGCC TATTGCGAAT
5701  AACCGTTCAG TTTTTACCAT CTCCCCAAAT GAATTGAAGG TTATACTTCA
5751  AAGTAATAAA GTAGTTTCTT CTCCCGTATC GATGACTCCT GATGGCCAAT
5801  ATATGCGGAC TGTCGATGTA GGAAAAGTTA TTGGTACTAC TTCTATTAAA
5851  GAAGGTGGAC AACCCACAAC TACAATTAAA GTATTTACAG ATAAGTCAGG
5901  AAATTTGATT ACTACATACC CAGTAAAAGG AAACTAA
```

This corresponds to the amino acid sequence <SEQ ID 60; ORF114-1>:

```
1     MNKGLHRIIF SKKHSTHVAV AETANSQGKG KQAGSSVSVS LKTSGDLCGK
51    LKTTLKTLVC SLVSLSMVLP AHAQITTDKS APKNQQVVIL KTNTGAPLVN
101   IQTPNGRGLS HNRYTQFDVD NKGAVLNNDR NNNPFVVKGS AQLILNEVRG
151   TASKLNGIVT VGGQKADVII ANPNGITVNG GGFKNVGRGI LTTGAPQIGK
201   DGALTGFDVR QGTLTVGAAG WNDKGGADYT GVLARAVALQ GKLQGKNLAV
251   STGPQKVDYA SGEISAGTAA GTKPTIALDT AALGGMYADS ITLIANEKGV
301   GVKNAGTLEA AKQLIVTSSG RIENSGRIAT TADGTEASPT YLSIETTEKG
351   AAGTFISNGG RIESKGLLVI ETGEDISLRN GAVVQNNGSR PATTVLNAGH
401   NLVIESKTNV NNAKGPATLS ADGRTVIKEA SIQTGTTVYS SSKGNAELGN
451   NTRITGADVT VLSNGTISSS AVIDAKDTAH IEAGKPLSLE ASTVTSDIRL
501   NGGSIKGGKQ LALLADDNIT AKTTNLNTPG NLYVHTGKDL NLNVDKDLSA
551   ASIHLKSDNA AHITGTSKTL TASKDMGVEA GSLNVTNTNL RTNSGNLHIQ
601   AAKGNIQLRN TKLNAAKALE TTALQGNIVS DGLHAVSADG HVSLLANGNA
651   DFTGHNTLTA KADVNAGSVG KGRLKADNTN ITSSSGDITL VAGNGIQLGD
701   GKQRNSINGK HISIKNNGGN ADLKNLNVHA KSGALNIHSD RALSIENTKL
751   ESTHNTHLNA QHERVTLNQV DAYAHRHLSI TGSQIWQNDK LPSANKLVAN
851   AELKPLAGRL NIEAGSGTLT IEPANRISAH TDLSIKTGGK LLLSAKGGNA
901   GAPSAQVSSL EAKGNIRLVT GETDLRGSKI TAGKNLVVAT TKGKLNIEAV
951   NNSFSNYFPT QKAAELNQKS KELEQQIAQL KKSSPKSKLI PTLQEERDRL
1001  AFYIQAINKE VKGKKPKGKE YLQAKLSAQN IDLISAQGIE ISGSDITASK
```

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1051  KLNLHAAGVL PKAADSEAAA ILIDGITDQY EIGKPTYKSH YDKAALNKPS
1101  RLTGRTGVSI HAAAALDDAR IIIGASEIKA PSGSIDIKAH SDIVLEAGQN
1151  DAYTFLKTKG KSGKIIRKTK FTSTRDHLIM PAPVELTANG ITLQAGGNIE
1201  ANTTRFNAPA GKVTLVAGEE LQLLAEEGIH KHELDVQKSR RFIGIKVGKS
1251  NYSKNELNET KLPVRVVAQT AATRSGWDTV LEGTEFKTTL AGADIQAGVG
1301  EKARADAKII LKGIVNRIQS EEKLETNSTV WQKQAGRGST IETLKLPSFE
1351  SPTPPKLTAP GGYIVDIPKG NLKTEIEKLA KQPEYAYLKQ LQVAKNVNWN
1401  QVQLAYDKWD YKQEGLTRAG AAIVTIIVTA LTYGYGATAA GGVAASGSST
1451  AAAAGTAATT TAAATTVSTA TAMQTAALAS LYSQAAVSII NNKGDVGKAL
1501  KDLGTSDTVK QIVTSALTAG ALNQMGADIA QLNSKVRTEL FSSTGNQTIA
1551  NLGGRLATNL SNAGISAGIN TAVNGGSLKD NLGNAALGAL VNSFQGEAAS
1601  KIKTTFSDDY VAKQFAHALA GCVSGLVQGK CKDGAIGAAV GEIVADSMLG
1651  GRNPATLSDA EKHKVISYSK IIAGSVAALN GGDVNTAANA AEVAVVNNAL
1701  NFDSTPTNAK KHQPQKPDKT ALEKIIQGIM PAHAAGAMTN PQDKDAAIWI
1751  SNIRNGITGP IVITSYGVYA AGWTAPLIGT AGKLAISTCM ANPSGCTVMV
1801  TQAAEAGAGI ATGAVTVGNA WEAPVGALSK AKAAKQAIPT QTVKELDGLL
1851  QESKNIGAVN TRINIANSTT RYTPMRQTGQ PVSAGFEHVL EGHFHRPIAN
1901  NRSVFTISPN ELKVILQSNK VVSSPVSMTP DGQYMRTVDV GKVIGTTSIK
1951  EGGQPTTTIK VFTDKSGNLI TTYPVKGN*
```

Computer analysis of this amino acid sequence predicts a transmembrane region and also gives the following results:

<u>Homology with a predicted ORF from *N.meningitidis* (strain A)</u>

ORF114 shows 91.9% identity over a 284aa overlap with an ORF (ORF114a) from strain A of *N. meningitidis*:

```
                                     10        20        30        40
orf114.pep                   AVAETANSQGKGKQAGSSVSVSLKTSGDLCGKLKTTLKTLVC
                             ||||||||||||||||||||||||||||||||||||||||||
orf114a      MNKGLHRIIFSKKHSTMVAVAETANSQGKGKQAGSSVSVSLKTSGDLCGKLKTTLKTLVC
                     10        20        30        40        50        60

                   50        60        70        80        90       100
orf114.pep   SLVSLSMVLPAHAQITTDKSAPKNQQVVILKTNTGAPLVNIQTPNGRGLSHNRXYAFDVD
             |||||||      ||||||||||| ||||||||||||||||||||||||||||   ||||
orf114a      SLVSLSMXXXXXXQITTDKSAPKNXQVVILKTNTGAPLVNIQTPNGRGLSHNRYTQFDVD
                     70        80        90       100       110       120

                  110       120       130       140       150       160
orf114.pep   NKGAVLNNDRNNNPFVVKGSAQLILNEVRGTASKLNGIVTVGGQKADVIIANPNGITVNG
             ||||||||||||||:|||||||||||||||||||||||||||||||||||||||||||||
orf114a      NKGAVLNNDRNNNPFLVKGSAQLILNEVRGTASKLNGIVTVGGQKADVIIANPNGITVNG
                    130       140       150       160       170       180

                  170       180       190       200       210       220
orf114.pep   GGFKNVGRGILTTGAPQIGKDGALTGFDVVKAHWTVXAAGWNDKGGAXYTGVLARAVALQ
             |||||||||||| ||||||||||||||||| ::  || |||||||||| |||||||||||
orf114a      GGFKNVGRGILTIGAPQIGKDGALTGFDVRQGTLTVGAAGWNDKGGADYTGVLARAVALQ
                    190       200       210       220       230       240

                  230       240       250       260       270       280
orf114.pep   GKXXGKXLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIANEKGV
             ||  || |||||||||||||||||||||||||||||||||||||||||||||||||| ||||
orf114a      GKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEKGV
                    250       260       270       280       290       300

orf114.pep   GVX
             ||
orf114a      GVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXGTFISNGG
                    310       320       330       340       350       360
```


The complete length ORF114a nucleotide sequence <SEQ ID 61> is:

```
   1  ATGAATAAAG GTTTACATCG CATTATCTTT AGTAAAAAGC ACAGCACCAT
  51  GGTTGCAGTA GCCGAAACTG CCAACAGCCA GGGCAAAGGT AAACAGGCAG
 101  GCAGTTCGGT TTCTGTTTCA CTGAAAACTT CAGGCGACCT TTGCGGCAAA
 151  CTCAAAACCA CCCTTAAAAC CTTGGTCTGC TCTTTGGTTT CCCTGAGTAT
 201  GGNATTNCNN NNCNNTNCCC AAATTACCAC CGACAAATCA GCACCTAAAA
 251  ACCANCAGGT CGTTATCCTT AAAACCAACA CTGGTGCCCC CTTGGTGAAT
 301  ATCCAAACTC CGAATGGACG CGGATTGAGC CACAACCGCT ATACGCAGTT
 351  TGATGTTGAC AACAAAGGGG CAGTGTTAAA CAACGACCGT AACAATAATC
 401  CGTTTCTGGT CAAAGGCAGT GCGCAATTGA TTTTGAACGA GGTACGCGGT
 451  ACGGCTAGCA AACTCAACGG CATCGTTACC GTAGGCGGTC AAAAGGCCGA
 501  CGTGATTATT GCCAACCCCA ACGGCATTAC CGTTAATGGC GGCGGCTTTA
 551  AAAATGTCGG TCGGGGCATC TTAACTATCG GTGCGCCCCA AATCGGCAAA
 601  GACGGTGCAC TGACAGGATT TGATGTGCGT CAAGGCACAT TGACCGTAGG
 651  AGCAGCAGGT TGGAATGATA AAGGCGGAGC CGACTACACC GGGGTACTTG
 701  CTCGTGCAGT TGCTTTGCAG GGGAAATTAC AGGGTAAAAA CCTGGCGGTT
 751  TCTACCGGTC CTCAGAAAGT AGATTACGCC AGCGGCGAAA TCAGTGCAGG
 801  TACGGCAGCG GGTACGAAAC CGACTATTGC CCTTGATACT GCCGCACTGG
 851  GCGGTATGTA CGCCGACAGC ATCACACTGA TTGCCANTGA AAAAGGCGTA
 901  GGCGTCAAAA ATGCCGGCAC ACTCGAAGCG GCCAAGCAAT TGATTGTGAC
 951  TTCGTCAGGC CGCATTGAAA ACAGCGGCCG CATCGCCACC ACTGCCGACG
1001  GCACCGAAGC TTCACCGACT TATCTNNCNA TCGAAACCAC CGAAAAAGGA
1051  GCNNCAGGCA CATTTATCTC CAATGGTGGT CGGATCGAGA GCAAAGGCTT
1101  ATTGGTTATT GAGACGGGAG AAGATATCAN CTTGCGTAAC GGAGCCGTGG
1151  TGCAGAATAA CGGCAGTCGC CCAGCTACCA CGGTATTAAA TGCTGGTCAT
1201  AATTTGGTGA TTGAGAGTAA AACTAATGTG AACAATGCCA AAGGCTCGNC
1251  TAATCTGTCG GCCGGCGGTC GTACTACGAT CAATGATGCT ACTATTCAAG
1301  CGGGCAGTTC CGTGTACAGC TCCACCAAAG GCGATACTGA NTTGGGTGAA
1351  AATACCCGTA TTATTGCTGA AAACGTAACC GTATTATCTA ACGGTAGTAT
1401  TGGCAGTGCT GCTGTAATTG AGGCTAAAGA CACTGCACAC ATTGAATCGG
1451  GCAAACCGCT TTCTTTAGAA ACCTCGACCG TTGCCTCCAA CATCCGTTTG
1501  AACAACGGTA ACATTAAAGG CGGAAAGCAG CTTGCTTTAC TGGCAGACGA
1551  TAACATTACT GCCAAAACTA CCAATCTGAA TACTCCCGGC AATCTGTATG
1601  TTCATACAGG TAAAGATCTG AATTTGAATG TTGATAAAGA TTTGTCTGCC
1651  GCCAGCATCC ATTTGAAATC GGATAACGCT GCCCATATTA CCGGCACCAG
1701  TAAAACCCTC ACTGCCTCAA AAGACATGGG TGTGGAGGCA GGCTTGCTGA
1751  ATGTTACCAA TACCAATCTG CGTACCAACT CGGGTAATCT GCACATTCAG
1801  GCAGCCAAAG GCAATATTCA GCTTCGCAAT ACCAAGCTGA ACGCAGCCAA
1851  GGCTCTCGAA ACCACCGCAT TGCAGGGCAA TATCGTTTCA GACGGCCTTC
1901  ATGCTGTTTC TGCAGACGGT CATGTATCCT TATTGGCCAA CGGTAATGCC
1951  GACTTTACCG GTCACAATAC CCTGACAGCC AAGGCCGATG TCNATGCAGG
2001  ATCGGTTGGT AAAGGCCGTC TGAAAGCAGA CAATACCAAT ATCACTTCAT
2051  CTTCAGGAGA TATTACGTTG GTTGCCGNNN NCGGTATTCA GCTTGGTGAC
2101  GGAAAACAAC GCAATTCAAT CAACGGAAAA CACATCAGCA TCAAAAACAA
2151  CGGTGGTAAT GCCGACTTAA AAAACCTTAA CGTCCATGCC AAAAGCGGGG
2201  CATTGAACAT TCATTCCGAC CGGGCATTGA GCATAGAAAA TACNAAGCTG
2251  GAGTCTACCC ATAATACGGA TCTTAATGCA CAACACGAGC GGGTAACGCT
2301  CAACCAAGTA GATGCCTACG CACACCGTCA TCTAAGCATT ANCGGCAGCC
2351  AGATTTGGCA AAACGACAAA CTGCCTTCTG CCAACAAGCT GGTGGCTAAC
2401  GGTGTATTGG CANTCAATGC GCGCTATTCC CAAATTGCCG ACAACACCAC
2451  GCTGAGAGCG GGTGCAATCA ACCTTACTGC CGGTACCGCC CTAGTCAAGC
2501  GCGGCAACAT CAATTGGAGT ACCGTTTCGA CCAAGACTTT GGAAGATAAT
2551  GCCGAATTAA AACCATTGGC CGGACGGCTG AATATTGAAG CAGGTAGCGG
2601  CACATTAACC ATCGAACCTG CCAACCGCAT CAGTGCGCAT ACCGACCTGA
2651  GCATCAAAAC AGGCGGAAAA TTGCTGTTGT CTGCAAAAGG AGGAAATGCA
2701  GGTGCGCNTA GTGCTCAAGT TTCCTCATTG GAAGCAAAAG GCAATATCCG
2751  TCTGGTTACA GGAGNAACAG ATTTAAGAGG TTCTAAAATT ACAGCCGGTA
2801  AAAACTTGGT TGTCGCCACC ACCAAAGGCA AGTTGAATAT CGAAGCCGTA
2851  AACAACTCAT TCAGCAATTA TTTTCNTACA CAAAAAGNGN NNGNNCTCAA
2901  CCAAAAATCC AAAGAATTGG AACAGCAGAT TGCGCAGTTG AAAAAAAGCT
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2951  CGCNTAAAAG CAAGCTGATT CCAACCCTGC AAGAAGAACG CGACCGTCTC
3001  GCTTTCTATA TTCAAGCCAT CAACAAGGAA GTTAAAGGTA AAAAACCCAA
3051  AGGCAAAGAA TACCTGCAAG CCAAGCTTTC TGCACAAAAT ATTGACTTGA
3101  TTTCCGCACA AGGCATCGAA ATCAGCGGTT CCGATATTAC CGCTTCCAAA
3151  AAACTGAACC TTCACGCCGC AGGCGTATTG CCAAAGGCAG CAGATTCAGA
3201  GGCGGCTGCT ATTCTGATTG ACGGCATAAC CGAGCAATAT GAAATTGGCA
3251  AGCCCACCTA CAAGAGTCAC TACGACAAAG CTGCTCTGAA CAAGCCTTCA
3301  CGTTTGACCG GACGTACGGG GGTAAGTATT CATGCAGCTG CGGCACTCGA
3351  TGATGCACGT ATTATTATCG GTGCATCCGA AATCAAAGCT CCCTCAGGCA
3401  GCATAGACAT CAAAGCCCAT AGTGATATTG TACTGGAGGC TGGACAAAAC
3451  GATGCCTATA CCTTCTTANA AACCAAAGGT AAAAGCGGCA NAATNATCAG
3501  AAAAACNAAG TTTACCAGCA CCNGCGANCA CCTGATTATG CCAGCCCCNG
3551  TCGAGCTGAC CGCCAACGGT ATCACGCTTC AGGCAGGCGG CAACATCGAA
3601  GCTAATACCA CCCGCTTCAA TGCCCCTGCA GGTAAAGTTA CCCTGGTTGC
3651  GGGTGAANAG NTGCAACTGC TGGCAGAAGA AGGCATCCAC AAGCACGAGT
3701  TGGATGTCCA AAAAAGCCGC CGCTTTATCG GCATCAAGGT AGGTNAGAGC
3751  AATTACAGTA AAAACGAACT GAACGAAACC AAATTGCCTG TCCGCGTCGT
3801  CGCCCAAANT GCAGCCACCC GTTCAGGCTG GGATACCGTG CTCGAAGGTA
3851  CCGAATTCAA AACCACGCTG GCCGGTGCCG ACATTCAGGC AGGTGTANGC
3901  GAAAAAGCCC GTGTCGATGC GAAAATTATC CTCAAAGGCA TTGTGAACCG
3951  TATCCAGTCG GAAGAAAAAT TAGAAACCAA CTCAACCGTA TGGCAGAAAC
4001  AGGCCGGACG CGGCAGCACT ATCGAAACGC TAAAACTGCC CAGCTTCGAA
4051  AGCCCTACTC CGCCCAAATT GTCCGCACCC GGCGGNTATA TCGTCGACAT
4101  TCCGAAAGGC AATCTGAAAA CCGAAATCGA AAAGCTGTCC AAACAGCCCG
4151  AGTATGCCTA TCTGAAACAG CTCCAAGTAG CGAAAAACAT CAACTGGAAT
4201  CAGGTGCAGC TTGCTTACGA CAGATGGGAC TACAAACAGG AGGGCTTAAC
4251  CGAAGCAGGT GCGGCGATTA TCGCACTGGC CGTTACCGTG GTCACCTCAG
4301  GCGCAGGAAC CGGAGCCGTA TTGGGATTAA ACGGTGCGNC CGCCGCCGCA
4351  ACCGATGCAG CATTCGCCTC TTTGGCCAGC CAGGCTTCCG TATCGTTCAT
4401  CAACAACAAA GGCGATGTCG GCAAAACCCT GAAAGAGCTG GGCAGAAGCA
4451  GCACGGTGAA AAATCTGGTG GTTGCCGCCG CTACCGCAGG CGTAGCCGAC
4501  AAAATCGGCG CTTCGGCACT GANCAATGTC AGCGATAAGC AGTGGATCAA
4551  CAACCTGACC GTCAACCTAG CCAATGNCGG GCAGTGCCGC ACTGAttaa
```

This encodes a protein having amino acid sequence <SEQ ID 62>:

```
   1  MNKGLHRIIF SKKHSTMVAV AETANSQGKG KQAGSSVSVS LKTSGDLCGK
  51  LKTTLKTLVC SLVSLSMXXX XXXQITTDKS APKNXQVVIL KTNTGAPLVN
 101  IQTPNGRGLS HNRYTQFDVD NKGAVLNNDR NNNPFLVKGS AQLILNEVRG
 151  TASKLNGIVT VGGQKADVII ANPNGITVNG GGFKNVGRGI LTIGAPQIGK
 201  DGALTGFDVR QGTLTVGAAG WNDKGGADYT GVLARAVALQ GKLQGKNLAV
 251  STGPQKVDYA SGEISAGTAA GTKPTIALDT AALGGMYADS ITLIAXEKGV
 301  GVKNAGTLEA AKQLIVTSSG RIENSGRIAT TADGTEASPT YLXIETTEKG
 351  AXGTFISNGG RIESKGLLVI ETGEDIXLRN GAVVQNNGSR PATTVLNAGH
 401  NLVIESKTNV NNAKGSXNLS AGGRTTINDA TIQAGSSVYS STKGDTXLGE
 451  NTRIIAENVT VLSNGSIGSA AVIEAKDTAH IESGKPLSLE TSTVASNIRL
 501  NNGNIKGGKQ LALLADDNIT AKTTNLNTPG NLYVHTGKDL NLNVDKDLSA
 551  ASIHLKSDNA AHITGTSKTL TASKDHGVEA GLLNVTNTNL RTNSGNLHIQ
 601  AAKGNIQLRN TKLNAAKALE TTALQGNIVS DGLHAVSADG HVSLLANGNA
 651  DFTGHNTLTA KADVXAGSVG KGRLKADNTN ITSSSGDITL VAXXGIQLGD
 701  GKQRNSINGK HISIKNNGGN ADLKNLNVHA KSGALNIHSD RALSIENTKL
 751  ESTHNTHLNA QHERVTLNQV DAYAHRHLSI XGSQIWQNDK LPSANKLVAN
 801  GVLAXNARYS QIADNTTLRA GAINLTAGTA LVKRGNINWS TVSTKTLEDN
 851  AELKPLAGRL NIEAGSGTLT IEPANRISAH TDLSIKTGGK LLLSAKGGNA
 901  GAXSAQVSSL EAKGNIRLVT GXTDLRGSKI TAGKNLVVAT TKGKLNIEAV
 951  NNSFSNYFXT QKXXXLNQKS KELEQQIAQL KKSSXKSKLI PTLQEERDRL
1001  AFYIQAINKE VKGKKPKGKE YLQAKLSAQN IDLISAQGIE ISGSDITASK
1051  KLNLHAAGVL PKAADSEAAA ILIDGITDQY EIGKPTYKSH YDKAALNKPS
1101  RLTGRTGVSI HAAAALDDAR IIIGASEIKA PSGSIDIKAH SDIVLEAGQN
1151  DAYTFLXTKG KSGXXIRKTK FTSTXXHLIM PAPVELTANG ITLQAGGNIE
1201  ANTTRFNAPA GKVTLVAGEX XQLLAEEGIH KHELDVQKSR RFIGIKVGXS
1251  NYSKNELNET KLPVRVVAQX AATRSGWDTV LEGTEFKTTL AGADIQAGVX
1301  EKARVDAKII LKGIVNRIQS EEKLETNSTV WQKQAGRGST IETLKLPSFE
1351  SPTPPKLSAP GGYIVDIPKG NLKTEIEKLS KQPEYAYLKQ LQVAKNINWN
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1401  QVQLAYDRWD YKQEGLTEAG AAIIALAVTV VTSGAGTGAV LGLNGAXAAA
1451  TDAAFASLAS QASVSFINNK GDVGKTLKEL GRSSTVKNLV VAAATAGVAD
1501  KIGASALXNV SDKQWINNLT VNLANXGQCR TD*
```

ORF114-1 and ORF114a show 89.8% identity in 1564 aa overlap

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orf114a.pep  MNKGLHRIIFSKKHSTMVAVAETANSQGKGKQAGSSVSVSLKTSGDLCGKLKTTLKTLVC
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf114-1     MNKGLHRIIFSKKHSTMVAVAETANSQGKGKQAGSSVSVSLKTSGDLCGKLKTTLKTLVC

orf114a.pep  SLVSLSMXXXXXXQITTDKSAPKNXQVVILKTNTGAPLVNIQTPNGRGLSHNRYTQFDVD
             |||||||      ||||||||||| |||||||||||||||||||||||||||||||||||
orf114-1     SLVSLSMVLPAHAQITTDKSAPKNQQVVILKTNTGAPLVNIQTPNGRGLSHNRYTQFDVD

orf114a.pep  NKGAVLNNDRNNNPFLVKGSAQLILNEVRGTASKLNGIVTVGGQKADVIIANPNGITVNG
             |||||||||||||||:||||||||||||||||||||||||||||||||||||||||||||
orf114-1     NKGAVLNNDRNNNPFVVKGSAQLILNEVRGTASKLNGIVTVGGQKADVIIANPNGITVNG

orf114a.pep  GGFKNVGRGILTIGAPQIGKDGALTGFDVRQGTLTVGAAGWNDKGGADYTGVLARAVALQ
             |||||||||||| |||||||||||||||||||||||||||||||||||||||||||||||
orf114-1     GGFKNVGRGILTTGAPQIGKDGALTGFDVRQGTLTVGAAGWNDKGGADYTGVLARAVALQ

orf114a.pep  GKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIAXEKGV
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||| ||||
orf114-1     GKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKPTIALDTAALGGMYADSITLIANEKGV

orf114a.pep  GVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLXIETTEKGAXGTFISNGG
             |||||||||||||||||||||||||||||||||||||||||| |||||||| ||||||||
orf114-1     GVKNAGTLEAAKQLIVTSSGRIENSGRIATTADGTEASPTYLSIETTEKGAAGTFISNGG

orf114a.pep  RIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNAGHNLVIESKTNVNNAKGSXNLS
             |||||||||||||||| ||||||||||||||||||||||||||||||||||||||  :||
orf114-1     RIESKGLLVIETGEDISLRNGAVVQNNGSRPATTVLNAGHNLVIESKTNVNNAKGPATLS

orf114a.pep  AGGRTTINDATIQAGSSVYSSTKGDTXLGENTRIIAENVTVLSNGSIGSAAVIEAKDTAH
             | |||:|::|:||:|::||||:||:: ||:|||| : :|||||||:|:|:|||:||||||
orf114-1     ADGRTVIKEASIQTGTTVYSSSKGNAELGNNTRITGADVTVLSNGTISSSAVIDAKDTAH

orf114a.pep  IESGKPLSLETSTVASNIRLNNGNIKGGKQLALLADDNITAKTTNLNTPGNLYVHTGKDL
             ||:|||||||:|||:|:||||:|:||||||||||||||||||||||||||||||||||||
orf114-1     IEAGKPLSLEASTVTSDIRLNGGSIKGGKQLALLADDNITAKTTNLNTPGNLYVHTGKDL

orf114a.pep  NLNVDKDLSAASIHLKSDNAAHITGTSKTLTASKDHGVEAGLLNVTNTNLRTNSGNLHIQ
             ||||||||||||||||||||||||||||||||||||||||| ||||||||||||||||||
orf114-1     NLNVDKDLSAASIHLKSDNAAHITGTSKTLTASKDHGVEAGSLNVTNTNLRTNSGNLHIQ

orf114a.pep  AAKGNIQLRNTKLNAAKALETTALQGNIVSDGLHAVSADGHVSLLANGNADFTGHNTLTA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf114-1     AAKGNIQLRNTKLNAAKALETTALQGNIVSDGLHAVSADGHVSLLANGNADFTGHNTLTA

orf114a.pep  KADVXAGSVGKGRLKADNTNITSSSGDITLVAXXGIQLGDGKQRNSINGKHISIKNNGGN
             |||| ||||||||||||||||||||||||||  ||||||||||||||||||||||||||
orf114-1     KADVNAGSVGKGRLKADNTNITSSSGDITLVAGNGIQLGDGKQRNSINGKHISIKNNGGN

orf114a.pep  ADLKNLNVHAKSGALNIHSDRALSIENTKLESTHNTHLNAQHERVTLNQVDAYAHRHLSI
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf114-1     ADLKNLNVHAKSGALNIHSDRALSIENTKLESTHNTHLNAQHERVTLNQVDAYAHRHLSI

orf114a.pep  XGSQIWQNDKLPSANKLVANGVLAXNARYSQIADNTTLRAGAINLTAGTALVKRGNINWS
             :||||||||||||||||||||||| |||||||||||||||||||||||||||||||||||
orf114-1     TGSQIWQNDKLPSANKLVANGVLALNARYSQIADNTTLRAGAINLTAGTALVKRGNINWS

orf114a.pep  TVSTKTLEDNAELKPLAGRLNIEAGSGTLTIEPANRISAHTDLSIKTGGKLLLSAKGGNA
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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```
orf114-1      TVSTKTLEDNAELKPLAGRLNIEAGSGTLTIEPANRISAHTDLSIKTGGKLLLSAKGGNA

orf114a.pep   GAXSAQVSSLEAKGNIRLVTGXTDLRGSKITAGKNLVVATTKGKLNIEAVNNSFSNYFXT
              || |||||||||||||||||| ||||||||||||||||||||||||||||||||||||| |
orf114-1      GAPSAQVSSLEAKGNIRLVTGETDLRGSKITAGKNLVVATTKGKLNIEAVNNSFSNYFPT

orf114a.pep   QKXXXLNQKSKELEQQIAQLKKSSXKSKLIPTLQEERDRLAFYIQAINKEVKGKKPKGKE
              ||   |||||||||||||||||||| |||||||||||||||||||||||||||||||||||
orf114-1      QKAAELNQKSKELEQQIAQLKKSSPKSKLIPTLQEERDRLAFYIQAINKEVKGKKPKGKE

orf114a.pep   YLQAKLSAQNIDLISAQGIEISGSDITASKKLNLHAAGVLPKAADSEAAAILIDGITDQY
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf114-1      YLQAKLSAQNIDLISAQGIEISGSDITASKKLNLHAAGVLPKAADSEAAAILIDGITDQY

orf114a.pep   EIGKPTYKSHYDKAALNKPSRLTGRTGVSIHAAAALDDARIIIGASEIKAPSGSIDIKAH
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf114-1      EIGKPTYKSHYDKAALNKPSRLTGRTGVSIHAAAALDDARIIIGASEIKAPSGSIDIKAH

orf114a.pep   SDIVLEAGQNDAYTFLXTKGKSGXXIRKTKFTSTXXHLIMPAPVELTANGITLQAGGNIE
              ||||||||||||||||| |||||  ||||||||||  |||||||||||||||||||||||
orf114-1      SDIVLEAGQNDAYTFLKTKGKSGKIIRKTKFTSTRDHLIMPAPVELTANGITLQAGGNIE

orf114a.pep   ANTTRFNAPAGKVTLVAGEXXQLLAEEGIHKHELDVQKSRRFIGIKVGXSNYSKNELNET
              ||||||||||||||||||||  ||||||||||||||||||||||||||| |||||||||||
orf114-1      ANTTRFNAPAGKVTLVAGEELQLLAEEGIHKHELDVQKSRRFIGIKVGKSNYSKNELNET

orf114a.pep   KLPVRVVAQXAATRSGWDTVLEGTEFKTTLAGADIQAGVXEKARVDAKIILKGIVNRIQS
              |||||||||:||||||||||||||||||||||||||||||| ||||:||||||||||||||
orf114-1      KLPVRVVAQTAATRSGWDTVLEGTEFKTTLAGADIQAGVGEKARADAKIILKGIVNRIQS

orf114a.pep   EEKLETNSTVWQKQAGRGSTIETLKLPSFESPTPPKLSAPGGYIVDIPKGNLKTEIEKLS
              |||||||||||||||||||||||||||||||||||||:||||||||||||||||||||||:
orf114-1      EEKLETNSTVWQKQAGRGSTIETLKLPSFESPTPPKLTAPGGYIVDIPKGNLKTEIEKLA

orf114a.pep   KQPEYAYLKQLQVAKNINWNQVQLAYDRWDYKQEGLTEAGAAIIALAVTVVTSGAGTGAV
              ||||||||||||||||:||||||||||:||||||||:|||||::: ||::| | |: |:
orf114-1      KQPEYAYLKQLQVAKNVNWNQVQLAYDKWDYKQEGLTRAGAAIVTIIVTALTYGYGATAA

orf114a.pep   LGLNGA--------------XAAATD---------AAFASLASQASVSFINNKGDVGKTL 1477
               |: ::              :||||          ||:||| |||:||:|||||||||:|
orf114-1      GGVAASGSSTAAAAGTAATTTAAATTVSTATAMQTAALASLYSQAAVSIINNKGDVGKAL 1500

orf114a.pep   KELGRSSTVKNLVVAAATAGVADKIGA----------SALXNVSDKQNINNL----TVNL 1523
              |:|| |:|||::|::| ||||: ::||          : | : : :| | ||    ::||
orf114-1      KDLGTSDTVKQIVTSALTAGALNQHGADIAQLNSKVRTELFSSTGNQTIANLGGRLATNL 1560

orf114a.pep   ANXGQCRTDX
              :| |
orf114-1      SNAGISAGINTAVN...
```

<u>Homology with pspA putative secreted protein of *N.meningitidis* (accession number AF030941)</u>

ORF114 and pspA protein show 36% aa identity in 302aa overlap:

```
Orf114: 1    AVAETANSQGKGKQAGSSVSVSL----KTSGDXXXXXXXXXXXXXXXXXXXXXXXPAHAQ 56
             AVAE  +  GK  Q   + SV +      S                        PA A
pspA:   19   AVAENVHRDGKSHQDSEAASVRVTGAASVSSARAAFGFRHAAFSVMLALGVAAFSPAPAS 78

Orf114: 57   -ITTDKSAPKNQQVVILKTNTGAPLVNIQTPNGRGLSHNRXYAFDVDHKGAVLNNDRNN- 114
              I  DKSAPKNQQ VIL+T  G P VNIQTP+ +G+S NR    FDVD KG +LNN R+N
pspA:   79   GIIADKSAPKNQQAVILQTANGLPQVNIQTPSSQGVSVNRFKQFDVDEKGVILNNSRSNT 138
```



```
Orf114: 115 ----------NPFVVKGSAQLILNEV-RGTASKLNGIVTVGGQKADVIIANPNGITVNGG 163
                      NP + +G A++I+N++     S LNG + VGG++A+V++ANP+GI VNGG
pspA:   139 QTQLGGWIQGNPHLARGEARVIVNQIDSSNPSLLNGYIEVGGKRAEVVVANPSGIRVNGG 198

Orf114: 164 GFKNVGRGILTTGAPQIGKDGALTGFDVVKAHWTVXAAGWNDKGGAXYTGVLARAVALQG 223
            G  N    LT+G P +  +G LTGFDV       +  G  D   A YT +L+RA  +
pspA:   199 GLINAASVTLTSGVPVL-NNGNLTGFDVSSGKVVIGGKGL-DTSDADYTRILSRAAEINA 256

Orf114: 224 KXXGKXLAVSTGPQKVDYASGEISAGTAAGTK----PTIALDTAALGGHYADSITLIANE 279
                GK + V +G  K+D+        +A  +      PT+A+DTA LGGHYAD ITLI+ +
pspA:   257 GVWGKDVKVVSGKNKLDFDGSLAKTASAPSSSDSVTPTVAIDTATLGGHYADKITLISTD 316

Orf114: 280 KG 281
             G
pspA:   317 NG 318
```

ORF114a is also homologous to pspA:

```
gi|2623258 (AF030941) putative secreted protein [Neisseria meningitidis] Length
= 2273
 Score =  261 bits (659), Expect = 3e-68
 Identities = 203/663 (30%), Positives = 314/663 (46%), Gaps = 76/663 (11%)

Query: 1   MNKGLHRIIFSKKHSTMVAVAETANSQGKGKQAGSSVSVSLK-----TSGDXXXXXXXXX 55
            MNK  +++IF+KK S M+AVAE  +  GK  Q   + SV +       +S
Sbjct: 1   MNKRCYKVIFNKKRSCMMAVAENVHRDGKSMQDSEAASVRVTGAASVSSARAAFGFRMAA 60

Query: 56  XXXXXXXXXXXXXXXXXQITTDKSAPKNXQVVILKTNTGAPLVNIQTPNGRGLSHNRYT 115
                             I  DKSAPKN Q VIL+T  G P VNIQTP+ +G+S NR+
Sbjct: 61  FSVMLALGVAAFSPAPASGIIADKSAPKNQQAVILQTANGLPQVNIQTPSSQGVSVNRFK 120

Query: 116 QFDVDNKGAVLNNDRNN-----------NPFLVKGSAQLILNEV-RGTASKLNGIVTVGG 163
            QFDVD KG +LNN R+N           NP L +G A++I+N++     S LNG + VGG
Sbjct: 121 QFDVDEKGVILNNSRSNTQTQLGGWIQGNPHLARGEARVIVNQIDSSNPSLLNGYIEVGG 180

Query: 164 QKADVIIANPNGITVNGGGFKNVGRGILTIGAPQIGKDGALTGFDVRQGTLTVGAAGWND 223
            ++A+V++ANP+GI VNGGG  N    LT G P +  +G LTGFDV  G + +G  G D
Sbjct: 181 KRAEVVVANPSGIRVNGGGLINAASVTLTSGVPVL-NNGNLTGFDVSSGKVVIGGKGL-D 238

Query: 224 KGGADYTGVLARAVALQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTK----PTIALD 279
              ADYT +L+RA  +   + GK++ V +G  K+D+        +A  +      PT+A+D
Sbjct: 239 TSDADYTRILSRAAEINAGVWGKDVKVVSGKNKLDFDGSLAKTASAPSSSDSVTPTVAID 298

Query: 280 TAALGGHYADSITLIAXEKGVGVKNAGTLEAAK-QLIVTSSGRIENSGRIATTADGTEAS 338
            TA LGGHYAD ITLI+ + G  ++N G + AA   + +++ G++ NSG I        +A+
Sbjct: 299 TATLGGHYADKITLISTDNGAVIRNKGRIFAATGGVTLSADGKLSNSGSI-------DAA 351

Query: 339 PTYLXIETTEKGAXGTFISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSRPATTVLNA 398
              +  +T +        +  G I S    V++  + I  + G +    GS     + +
Sbjct: 352 EITISAQTVD--------NRQGFIRSGKGSVLKVSDGINNQAGLI----GSAGLLDIRDT 399

Query: 399 GHNLVIESKTNVNNAKGS----XNLSAGGRTTINDATIQAGSSVYSSTKGDTXLGENTRI 454
            G     +S  ++NN  G+     ++S   ++  ND  + A   V S + D   G+
Sbjct: 400 G-----KSSLHINNTDGTIIAGKDVSLQAKSLDNDGILTAARDV-SVSLHDDFAGKRDIE 453

Query: 455 IAENVTVLSNGSIGSAAVIEAKDTAHIESGKPLSLETSTVASNIRLNNGNIKGGKQLALL 514
                +T  + G + +  +I+A DT  + + +  + +  + S R       G      L+
Sbjct: 454 AGRTLTFSTQGRLKNTRIIQAGDTVSLTAAQIDNTVSGKIQSGNRTGLNGKNGITNRGLI 513

Query: 515 ADDNIT-----AKTTNLNTPGNLYVHTGKDLNLNVDKDLSAASIHLKSDNAAHITGTSKT 569
             + IT     AK+ N  T G +Y   G  + +  D L+              AA
Sbjct: 514 NSNGITLLQTEAKSDNAGT-GRIY---GSRVAVEADTLLNREETVNGETKAA-------V 562

Query: 570 LTASKDMGVEAGXXXXXXXXXXXSGNLHIQAA---KGNIQLRNTKL-NAAKALETTALQ 625
```

```
                + A + + + A                  SG+LHI +A      +Q  NT L N + A+E++
Sbjct: 563 IAARERLDIGAREIENREAALLSSSGDLHIGSALNGSRQVQGANTSLHNRSAAIESS--- 619

Query: 626 GNI 628
            GNI
Sbjct: 620 GNI 622


 Score = 37.5 bits (85), Expect = 0.53
 Identities = 87/432 (20%), Positives = 159/432 (36%), Gaps = 62/432 (14%)

Query: 239  LQGKLQGKNLAVSTGPQKVDYASGEISAGTAAGTKETIALDTAALGGHYADSITLIAXEK 298
             LQG LQGKN+  + G    + +G I A  A  K         A  + + S T     +
Sbjct: 1023 LQGDLQGKNIFAAAGSDITN--TGSIGAENALLLK--------ASNNIESRSETRSNQNE 1072

Query: 299  GVGVKNAGTLEAAKQLIVTSSGRI--ENSGRIATTADGTEASPTYLXIETTEKGAXG-TF 355
                V+N G + A  L    +G +  +    I  TA              E T +  G T
Sbjct: 1073 QGSVRNIGRV-AGIYLTGRQNGSVLLDAGNNIVLTAS-----------ELTNQSEDGQTV 1120

Query: 356  ISNGGRIESKGLLVIETGEDIXLRNGAVVQNNGSREATTVLNAGHNLVIESK-------T 408
             ++ GG I S    +      I   +  V++    +   +T+   G NL + +K
Sbjct: 1121 LNAGGDIRSDTTGISRNQNTIFDSDNYVIRKEQNEVGSTIRTRG-NLSLNAKGDIRIRAA 1179

Query: 409  NVNNAKGSXNLSAGGRTTINDATIQAGSS--------VYSSTKGDTXLGENTRIIAENVT 460
              V + +G   L+AG        D  ++AG +          Y+    G     + TR +
Sbjct: 1180 EVGSEQGRLKLAAG-----RDIKVEAGKAHTETEDALKYTGRSGGGIKQKMTRHLKNQNG 1234

Query: 461  VLSNGSIGSAAVIEAKDTAHIESGKPLSLETSTVASNIRLNNGNIKGGKQLALLADDNIT 520
                +G++      +I            +G +   +   T+ S    NN  +K  +   A+ N
Sbjct: 1235 QAVSGTLDGKEIILVSGRDITVTGSNIIADNHTILS--AKNNIVLKAAETRSRSAENNKK 1292

Query: 521  AKTTNLNTPG-NLYVHTGKDLNLNVDKDLSAASIHEKSDN-------AAHITGTSNTLTA 572
              K+   + G       + KD    N  + +S        + S N          N T T  T+++
Sbjct: 1293 EKSGLNGSGGIGFTAGSKKDTQTNRSETVSHTESVVGSLNGNTLISAGKHYTQTGSTISS 1352

Query: 573  SK-DNGVEAGXXXXXXXXXXXXSGNLHIQAAKG-----NIQLRNTKLNAAKALETTALQG 626
              + D+G+ +G                   +   +   KG      ++ + NT + A  A++      G
Sbjct: 1353 PQGDVGISSGKISIDAAQNRYSQESKQVYEQKGVTVAISVPVVNTVNGAVDAVKAVQTVG 1412

Query: 627  NIVSDGLHAVSA 638
                    +   ++A++A
Sbjct: 1413 KSKNSRVNAMAA 1424
```

Amino acids 1-1423 of ORF114-1 were cloned in the pGex vector and expressed in *E.coli*, as described above. GST-fusion expression was visible using SDS-PAGE, and Figure 5 shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF114-1.

Based on these results, including the homology with the putative secreted protein of *N.meningitidis* and on the presence of a transmembrane domain, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 14

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 63>


```
   1  ..CGCTTCATTC ATGATGAAGC AGTCGGCAGC AACATCGGCG GCGGCAAAAT
  51    GATTGTTGCA GCCGGGCAGG ATATCAATGT ACGCGGCAnA AGCCTTATTT
 101    CTGATAAGGG CATTGTTTTA AAAGCAGGAC ACGACATCGA TATTTCTACT
 151    GCCCATAATC GCTATACCGG CAATGAATAC CACGAGAGCA wAAAwTCAGG
 201    CGTCATGGGT ACTGGCGGAT TGGGCTTTAC TATCGGTAAC CGGAAAACTA
 251    CCGATGACAC TGATCGTACC AATATTGTsC ATACAGGCAG CATTATAGGC
 301    AGCCTGAaTG GAGACACCGT TACAGTTGCA GGAAACCGCT ACCGACAAAC
 351    CGGCAGTACC GTCTCCAGCC CCGAGGGGCG CAATACCGTC ACAGCCAAAw
 401    GCATAGATGT AGAGTTCGCA AACAACCGGT ATGCCACTGA CTACGcCCAT
 451    ACCCAgGGAA CAAAAAGGCC TTACCGTCGC CCTCAATGTC CCGGTTGTCC
 501    AAGCTGCACA AAACTTCATA CAAGCAGCCC AAAATGTGGG CAAAAGTAAA
 551    AATAAACGCG TTAATGCCAT GGCTGCAGCC AATGCTGCAT GGCAGAGTTA
 601    TCAAGCAACC CAACAAATGC AACAATTTGC TCCAAGCAGC AGTGCGGGAC
 651    AAGGTCAAAA CTACAATCAA AGCCCCAGTA TCAGTGTGTC CATTAC.TAC
 701    GGCGAACAGA AAAGTCGTAA CGAGCAAAAA AGACATTACA CCGAAgCGGC
 751    AgCAAGTCAA ATTATCGGCA AAGGGCAAAC CACACTTGCG GCAACAGGAA
 801    GTGGGGAGCA GTCCAATATC AATATTACAG GTTCCGATGT CATCGGCCAT
 851    GCAGGTACTC C.CTCATTGC CGACAACCAT ATCAGACTCC AATCTGCCAA
 901    ACAGGACGGC AGCGAGCAAA GCAAAAACAA AAGCAGTGGT TGGAATGCAG
 951    GCGTACGTnn CAAAATAGGC AACGGCATCA GGTTTGGAAT TACCGCCGGA
1001    GGAAATATCG GTAAAGGTAA AGAGCAAGGG GGAAGTACTA CCCACCGCCA
1051    CACCCATGTC GGCAGCACAA CCGGCAAAAC TACCATCCGA AGCGGCGGGG
1101    GATACCACCC TCAAAGGTGT GCAGCTCATC GGCAAAGGCA TACAGGCAGA
1151    TACGCGCAAC CTGCATATAG AAAGTGTTCA AGATACTGAA ACCTATCAGA
1201    GCAAACAGCA AAACGGCAAT GTCCAAGTTt ACTGTCGGTT ACGGATTCAG
1251    TGCAAGCGGC AGTTACCGCC AAAGCAAAGT CAAAGCAGAC CATGCCTCCG
1301    TAACCGGGCA AAgCGGTATT TATGCCGGAG AAGACGGCTA TCAAATyAAA
1351    GTyAGAGACA ACACAGACCT yAAGGGCGGT ATCATCACGT CTAGCCAAAG
1401    CGCAGAAGAT AAGGGCAAAA ACCTTTTTCA GACGGCCACC CTTACTGCCA
1451    GCGACATTCA AAACCACAGC CGCTACGAAG GCAGAAGCTT CGGCATAGGC
1501    GGCAGTTTCG ACCTGAACGG CGGCTGGGAC GGCACGGTTA CCGACAAACA
1551    AGGCAGGCCT ACCGACAGGA TAAGCCCGGC AGCCGGCTAC GGCAGCGACG
1601    GAGACAGCAA AAACAGCACC ACCCGCAGCG GCGTCAACAC CCACAACATA
1651    CACATCACCG ACGAAGCGGG ACAACTTGCC CGAACAGGCA GGACTGCAAA
1701    AGAAACCGAA GCGCGTATCT ACACCGGCAT CGACACCGAA ACTGCGGATC
1751    AACACTCAGG CCATCTGAAA AACAGCTTCG AC...
```

This corresponds to the amino acid sequence <SEQ ID 64; ORF116>:

```
  1  ..RFIHDEAVGS NIGGGKMIVA AGQDINVRGX SLISDKGIVL KAGHDIDIST
 51    AHNRYTGNEY HESXXSGVMG TGGLGFTIGN RKTTDDTDRT NIVHTGSIIG
101    SLNGDTVTVA GNRYRQTGST VSSPEGRNTV TAKXIDVEFA NNRYATDYAH
151    TQEQKGLTVA LNVPVVQAAQ NFIQAAQNVG KSKNKRVNAM AAANAAWQSY
201    QATQQMQQFA PSSSAGQGQN YNQSPSISVS IXYGEQKSRN EQKRHYTEAA
251    ASQIIGKGQT TLAATGSGEQ SNINITGSDV IGHAGTXLIA DNHIRLQSAK
301    QDGSEQSKNK SSGWNAGVRX KIGNGIRFGI TAGGNIGKGK EQGGSTTHRH
351    THVGSTTGKT TIRSGGDTTL KGVQLIGKGI QADTRNLHIE SVQDTETYQS
401    KQQNGNVQVT VGYGFSASGS YRQSKVKADH ASVTGQSGIY AGEDGYQIKV
451    RDNTDLKGGI ITSSQSAEDK GKNLFQTATL TASDIQNHSR YEGRSFGIGG
501    SFDLNGGWDG TVTDKQGRPT DRISPAAGYG SDGDSKNSTT RSGVNTHNIH
551    ITDEAGQLAR TGRTAKETEA RIYTGIDTET ADQHSGHLKN SFD...
```

Computer analysis of this amino acid sequence gave the following results:

<u>Homology with pspA putative secreted protein of *N.meningitidis* (accession number AF030941)</u>

ORF116 and pspA protein show 38% aa identity in 502aa overlap:

```
Orf116: 6     EAVGSNIGGGKMIVAAGQDINVRGXSLISDKGIVLKAGHDIDISTAHNRYTGNEYHESXX 65
              +AV   + G ++I+ +G+DI V G ++I+D   +L A ++I +   A  R    E ++
PspA: 1  235  QAVSGTLDGKEIILVSGRDITVTGSNIIADNHTILSAKNNIVLKAAETRSRSAENNKKEK 1294
```

```
Orf116: 66   XXXXXXXXXXXXXXNRKXXXXXXRTNIVHTGSIIGSLNGDTVTVAGNRYRQTGSTVSSPE 125
                           ++K         + HT S++GSLNG+T+  AG  Y QTGST+SSP+
PspA:   1295 SGLMGSGGIGFTAGSKKDTQTNRSETVSHTESVVGSLNGNTLISAGKHYTQTGSTISSPQ 1354

Orf116: 126  GRNTVTAKXIDVEFANNRYATDYAHTQEQKGLTVALNVPXXXX---XXXXXXXXXXGKS 182
             G   +++  I ++ A NRY+ +       EQKG+TVA++VP                GKS
PspA:   1355 GDVGISSGKISIDAAQNRYSQESKQVYEQKGVTVAISVPVVNTVMGAVDAVKAVQTVGKS 1414

Orf116: 183  KNKRVXXXXXXXXXXWQSYQATQQMQQFA--PSSSAGQGQNYNQSPSISVSIXYGEQKSRN 240
             KN RV               +   +   +   A  P  +AGQG          ISVS+ YGEQK+ +
PspA:   1415 KNSRVNAMAAANALNKGVDSGVALYNAARNPKKAAGQG--------ISVSVTYGEQKNTS 1466

Orf116: 241  EQKRHYTEAAASQIIGKGQTTLAATGSGEQSHINITGSDVIGHAGTXLIADNHIRLQSAK 300
             E +   T+     +I G G+ +L A+G+G+ S I ITGSDV G  GT L A+N +++++A+
PspA:   1467 ESRIKGTQVQEGKITGGGKVSLTASGAGKDSRITITGSDVYGGKGTRLKAENAVQIEAAR 1526

Orf116: 301  QDGSEQSKNKSSGWNAGVRXKIGNGIRFGITAXXXXXXXXXXXXSTTHRHTHVGSTTGKT 360
             Q    E+S+NKS+G+NAGV    I  GI FG TA              T +R++H+GS   +T
PspA:   1527 QTHQERSENKSAGFNAGVAIAINKGISFGFTAGANYGKGYGNGDETAYRNSHIGSKDSQT 1586

Orf116: 361  TIRSGGDTTLKGVQLIGKGIQADTRNLHIESVQDTETYQSKQQNGNVQVTVGYGFSASGS 420
              I SGGDT +KG QL GKG+       +LHIES+QDT  ++ KQ+N + QVTVGYGFS  GS
PspA:   1587 AIESGGDTVIKGGQLKGKGVGVTAESLHIESLQDTAVFKGKQENVSAQVTVGYGFSVGGS 1646

Orf116: 421  YRQSKVKADHASVTGQSGIYAGEDGYQIKVRDNTDLKGGIITSSQSAEDKGKNLFQTATL 480
             Y +SK  +D+ASV  QSGI+AG DGY+I+V   T L G  + S     DK KNL +T+ +
PspA:   1647 YNRSKSSSDYASVNEQSGIFAGGDGYRIRVNGKTGLVGAAVVSD---ADKSKNLLKTSEI 1703

Orf116: 481  TASDIQNHSRYEGRSFGIGGSF 502
                DIQNH+      + G+ G F
PspA:   1704 WHKDIQNHASAAASALGLSGGF 1725
```

Based on homology with pspA, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 15

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 65>

```
  1  ..ACGACCGGCA GCCTCGGCGG CATACTGGCC GGCGGCGGCA CTTCCCTTGC
 51    CGCACCGTAT TTGGACAAAG CGGCGGAAAA CCTCGGTCCG GCGGGCAAAG
101    CGGCGGTCAA CGCACTGGGC GGTGCGGCCA TCGGCTATGC AACTGGTGGT
151    AGTGGTGGTG CTGTGGTGGG TGCGAATGTA GATTGGAACA ATAGGCAGCT
201    GCATCCGAAA GAAATGGCGT TGGCCGACAA ATATGCCGAA GCCCTCAAGC
251    GCGAAGTTGA AAAACGCGAA GGCAGAAAAA TCAGCAGCCA AGAAGCGGCA
301    ATGAGAATCC GCAGGCAGAT ATGCGTTGGG TGGACAAAGG TTCCCAAGAC
351    GGCTATACCG ACCAAAGCGT CATATCCCTT ATCGGAATGA
```

This corresponds to the amino acid sequence <SEQ ID 66; ORF118>:

```
  1  ..TTGSLGGILA GGGTSLAAPY LDKAAENLGP AGKAAVNALG GAAIGYATGG
 51    SGGAVVGANV DWNNRQLHPK EMALADKYAE ALKREVEKRE GRKISSQEAA
101    MRIRRQICVG WTKVPKTAIP TKASYPLSE*
```

Computer analysis of this amino acid sequence reveals two putative transmembrane domains.

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 16

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 67>

```
  1  ..CAATGCCGTC TGAAAAGCTC ACAATTTTAC AGACGGCATT TGTTATGCAA
 51    GTACATATAC AGATTCCCTA TATACTGCCC AGrkGCGTGC GTgGCTGAAG
101    ACACCCCCTA CGCTTGCTAT TTGrAACAGC TCCAAGTCAC CAAAGACGTC
151    AACTGGAACC AGGTACvACT GGCGTACGAC AAATGGGACT ATAAACAGGA
201    AGGCTTAACC GGAGCCGGAG CAGCGATTAT TGCGCTGGCT GTTACCGTGG
251    TTACTGCGGG CGCGGGAgCC GGAGCCGCAC TGGGcTTAAA CGGCGCGGCc
301    GCAGCGGCAA CCGATGCCGC ATTCGCCTCG CTGGCCAGCC AGGcTTCCGT
351    ATCGCTCATC AaCAACAAAG GCAATATCGG TAaCACCCTG AAAGAGCTGG
401    GCAGAAGCAG CACGGTGAAA AATCTGATGG TTGCCGTCGc tACCGCAgGC
451    GTagCcgaCA AAATCGGTGC TTCGGCACTG AACAATGTCA GCGATAAGCA
501    GTGGATCAAC AACCTGACCG TCAACCTGGC CAATGCGGGC AGTGCCGCAC
551    TGATTAATAC CGCTGTCAAC GGCGGCAGCc tgAAAGACAA TCTGGAAGCG
601    AATATCCTTG CGGCTTTGGT GAATACTGCG CATGGAGAAG CAGCCAGTAA
651    AATCAAACAG TTGGATCAGC ACTACATTAC CCACAAGATT GCCCaTGCCA
701    TAGCGGGCTG TGCGGCTGCG GCGGCGAATA AGGGCAAGTG TCAGGATGGT
751    GCGATAgGTG CGGCTGTGGG CGAGATAGTC GGGGAgGCTT TGACAAACGG
801    CAAAAATCCT GACACTTTGA CAGCTAAAgA ACGCGaACAG ATTTTGGCAT
851    ACAGCAAACT GGTTGCCGGT ACGGTAAGCG GTGTGGTCGG CGGCGATGTA
901    AATGCGGCGG CGAATGCGGC TGAGGTAGCG GTGAAAAATA ATCAGCTTAG
951    CGACAAAtGA
```

This corresponds to the amino acid sequence <SEQ ID 68; ORF41>:

```
  1  ..QCRLKSSQFY RRHLLQKYIY RFPIYCPXAC VAEDTPYACY LXQLQVTKDV
 51    NWNQVXLAYD KWDYKQEGLT GAGAAIIALA VTVVTAGAGA GAALGLNGAA
101    AAATDAAFAS LASQASVSLI NNKGNIGNTL KELGRSSTVK NLMVAVATAG
151    VADKIGASAL NNVSDKQWIN NLTVNLANAG SAALINTAVN GGSLKDNLEA
201    NILAALVNTA HGEAASKIKQ LDQHYITHKI AHAIAGCAAA AANKGKCQDG
251    AIGAAVGEIV GEALTNGKNP DTLTAKEREQ ILAYSKLVAG TVSGVVGGDV
301    NAAANAAEVA VKNNQLSDK*
```

Further work revealed the complete nucleotide sequence <SEQ ID 69>:

```
   1  ATGCAAGTAA ATATTCAGAT TCCCTATATA CTGCCCAGAT GCGTGCGTGC
  51  TGAAGACACC CCCTACGCTT GCTATTTGAA ACAGCTCCAA GTCACCAAAG
 101  ACGTCAACTG GAACCAGGTA CAACTGGCGT ACGACAAATG GGACTATAAA
 151  CAGGAAGGCT TAACCGGAGC CGGAGCAGCG ATTATTGCGC TGGCTGTTAC
 201  CGTGGTTACT GCGGGCGCGG GAGCCGGAGC CGCACTGGGC TTAAACGGCG
 251  CGGCCGCAGC GGCAACCGAT GCCGCATTCG CCTCGCTGGC CAGCCAGGCT
 301  TCCGTATCGC TCATCAACAA CAAAGGCAAT ATCGGTAACA CCCTGAAAGA
 351  GCTGGGCAGA AGCAGCACGG TGAAAAATCT GATGGTTGCC GTCGCTACCG
 401  CAGGCGTAGC CGACAAAATC GGTGCTTCGG CACTGAACAA TGTCAGCGAT
 451  AAGCAGTGGA TCAACAACCT GACCGTCAAC CTGGCCAATG CGGGCAGTGC
 501  CGCACTGATT AATACCGCTG TCAACGGCGG CAGCCTGAAA GACAATCTGG
 551  AAGCGAATAT CCTTGCGGCT TTGGTGAATA CTGCGCATGG AGAAGCAGCC
 601  AGTAAAATCA AACAGTTGGA TCAGCACTAC ATTACCCACA AGATTGCCCA
 651  TGCCATAGCG GGCTGTGCGG CTGCGGCGGC GAATAAGGGC AAGTGTCAGG
 701  ATGGTGCGAT AGGTGCGGCT GTGGGCGAGA TAGTCGGGGA GGCTTTGACA
 751  AACGGCAAAA ATCCTGACAC TTTGACAGCT AAAGAACGCG AACAGATTTT
 801  GGCATACAGC AAACTGGTTG CCGGTACGGT AAGCGGTGTG GTCGGCGGCG
 851  ATGTAAATGC GGCGGCGAAT GCGGCTGAGG TAGCGGTGAA AAATAATCAG
 901  CTTAGCGACA AAGAGGGTAG AGAATTTGAT AACGAAATGA CTGCATGCGC
 951  CAAACAGAAT AATCCTCAAC TGTGCAGAAA AAATACTGTA AAAAAGTATC
1001  AAAATGTTGC TGATAAAAGA CTTGCTGCTT CGATTGCAAT ATGTACGGAT
1051  ATATCCCGTA GTACTGAATG TAGAACAATC AGAAAACAAC ATTTGATCGA
1101  TAGTAGAAGC CTTCATTCAT CTTGGGAAGC AGGTCTAATT GGTAAAGATG
1151  ATGAATGGTA TAAATTATTC AGCAAATCTT ACACCCAAGC AGATTTGGCT
1201  TTACAGTCTT ATCATTTGAA TACTGCTGCT AAATCTTGGC TTCAATCGGG
```

```
1251  CAATACAAAG CCTTTATCCG AATGGATGTC CGACCAAGGT TATACACTTA
1301  TTTCAGGAGT TAATCCTAGA TTCATTCCAA TACCAAGAGG GTTTGTAAAA
1351  CAAAATACAC CTATTACTAA TGTCAAATAC CCGGAAGGCA TCAGTTTCGA
1401  TACAAACCTA AAAAGACATC TGGCAAATGC TGATGGTTTT AGTCAAAAAC
1451  AGGGCATTAA AGGAGCCCAT AACCGCACCA ATTTTATGGC AGAACTAAAT
1501  TCACGAGGAG GACGCGTAAA ATCTGAAACC CAAACTGATA TTGAAGGCAT
1551  TACCCGAATT AAATATGAGA TTCCTACACT AGACAGGACA GGTAAACCTG
1601  ATGGTGGATT TAAGGAAATT TCAAGTATAA AAACTGTTTA TAATCCTAAA
1651  AAATTTTCTG ATGATAAAAT ACTTCAAATG GCTCAAAATG CTGCTTCACA
1701  AGGATATTCA AAAGCCTCTA AAATTGCTCA AAATGAAAGA ACTAAATCAA
1751  TATCGGAAAG AAAAAATGTC ATTCAATTCT CAGAAACCTT TGACGGAATC
1801  AAATTTAGAT GATATTTTGA TGTAAATACA GGAAGAATTA CAAACATTCA
1851  CCCAGAATAA
```

This corresponds to the amino acid sequence <SEQ ID 70; ORF41-1>:

```
  1  MQVNIQIPYI LPRCVRAEDT PYACYLKQLQ VTKDVNWNQV QLAYDKWDYK
 51  QEGLTGAGAA IIALAVTVVT AGAGAGAALG LNGAAAAATD AAFASLASQA
101  SVSLINNKGN IGNTLKELGR SSTVKNLMVA VATAGVADKI GASALNNVSD
151  KQWINNLTVN LANAGSAALI NTAVNGGSLK DNLEANILAA LVNTAHGEAA
201  SKIKQLDQHY ITHKIAHAIA GCAAAAANKG KCQDGAIGAA VGEIVGEALT
251  NGKNPDTLTA KEREQILAYS KLVAGTVSGV VGGDVNAAAN AAEVAVKNNQ
301  LSDKEGREFD NEMTACAKQN NPQLCRKNTV KKYQNVADKR LAASIAICTD
351  ISRSTECRTI RKQHLIDSRS LHSSWEAGLI GKDDEWYKLF SKSYTQADLA
401  LQSYHLNTAA KSWLQSGNTK PLSEWMSDQG YTLISGVNPR FIPIPRGFVK
451  QNTPITNVKY PEGISFDTNL KRHLANADGF SQKQGIKGAH NRTNFMAELN
501  SRGGRVKSET QTDIEGITRI KYEIPTLDRT GKPDGGFKEI SSIKTVYNPK
551  KFSDDKILQM AQNAASQGYS KASKIAQNER TKSISERKNV IQFSETFDGI
601  KFRSYFDVNT GRITNIHPE*
```

Computer analysis of this amino acid sequence predicts a transmembrane domain, and homology with an ORF from *N.meningitidis* (strain A) was also found.

ORF41 shows 92.8% identity over a 279aa overlap with an ORF (ORF41a) from strain A of *N. meningitidis:*

```
                     10        20        30        40        50        60        69
orf41.pep   YRRHLLCKYIYRFPIYCPXACVAEDTPYACYLXQLQVTKDVNWNQVXLAYDKWDYKQEGL
                                              || ||||:|::||||| ||||:||||||||
orf41a                                        YLKQLQVAKNINWNQVQLAYDRWDYKQEGL
                                                      10        20        30

                     70        80        90       100       110       120       129
orf41.pep   TGAGAAIIALAVTVVTAGAGAGAALGLNGAAAAATDAAFASLASQASVSLINNKGNIGNT
            | ||||||||||||||:|||:||:|||||| |||||||||||||||||||:||||||::|:|
orf41a      TEAGAAIIALAVTVVTSGAGTGAVLGLNGAXAAATDAAFASLASQASVSFINNKGDVGKT
                    40        50        60        70        80        90

                    130       140       150       160       170       180       189
orf41.pep   LKELGRSSTVKNLMVAVATAGVADKIGASALNNVSDKQWINNLTVNLANAGSAALINTAV
            |||||||||||||:||:|||||||||||||||| ||||||||||||||||||||||||||||
orf41a      LKELGRSSTVKNLVVAAATAGVADKIGASALXNVSDKQWINNLTVNLANAGSAALINTAV
                    100       110       120       130       140       150

                    190       200       210       220       230       240       249
orf41.pep   NGGSLKDNLEANILAALVNTAHGEAASKIKQLDQHYITHKIAHAIAGCAAAAANKGKCQD
            ||||||| |||||||||||||||||||||||||||||:|||||||||||||||||||||||
orf41a      NGGSLKDXLEANILAALVNTAHGEAASKIKQLDQHYIVHKIAHAIAGCAAAAANKGKCQD
                    160       170       180       190       200       210
```



```
                    250       260       270       280       290       300       309
orf41.pep   GAIGAAVGEIVGEALTNGKNPDTLTAKEREQILAYSKLVAGTVSGVVGGDVNAAANAAEV
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf41a      GAIGAAVGEIVGEALTNGKNPDTLTAKEREQILAYSKLVAGTVSGVVGGDVNAAANAAEV
                    220       230       240       250       260       270

                    310       320
orf41.pep   AVKNNQLSDKX
            |||||||||
orf41a      AVKNNQLSDXEGREFDNEMTACAKQNXPQLCRKNTVKKYQNVADKRLAASIAICTDISRS
                    280       290       300       310       320       330
```

A partial ORF41a nucleotide sequence <SEQ ID 71> is:

```
   1  ..TATCTGAAAC AGCTCCAAGT AGCGAAAAAC ATCAACTGGA ATCAGGTGCA
  51    GCTTGCTTAC GACAGATGGG ACTACAAACA GGAGGGCTTA ACCGAAGCAG
 101    GTGCGGCGAT TATCGCACTG GCCGTTACCG TGGTCACCTC AGGCGCAGGA
 151    ACCGGAGCCG TATTGGGATT AAACGGTGCG NCCGCCGCCG CAACCGATGC
 201    AGCATTCGCC TCTTTGGCCA GCCAGGCTTC CGTATCGTTC ATCAACAACA
 251    AAGGCGATGT CGGCAAAACC CTGAAAGAGC TGGGCAGAAG CAGCACGGTG
 301    AAAAATCTGG TGGTTGCCGC CGCTACCGCA GGCGTAGCCG ACAAAATCGG
 351    CGCTTCGGCA CTGANCAATG TCAGCGATAA GCAGTGGATC AACAACCTGA
 401    CCGTCAACCT AGCCAATGCG GGCAGTGCCG CACTGATTAA TACCGCTGTC
 451    AACGGCGGCA GCCTGAAAGA CANTCTGGAA GCGAATATCC TTGCGGCTTT
 501    GGTCAATACC GCGCATGGAG AAGCAGCCAG TAAAATCAAA CAGTTGGATC
 551    AGCACTACAT AGTCCACAAG ATTGCCCATG CCATAGCGGG CTGTGCGGCA
 601    GCGGCGGCGA ATAAGGGCAA GTGTCAGGAT GGTGCGATAG GTGCGGCTGT
 651    GGGCGAGATA GTCGGGGAGG CTTTGACAAA CGGCAAAAAT CCTGACACTT
 701    TGACAGCTAA AGAACGCGAA CAGATTTTGG CATACAGCAA ACTGGTTGCC
 751    GGTACGGTAA GCGGTGTGGT CGGCGGCGAT GTAAATGCGG CGGCGAATGC
 801    GGCTGAGGTA GCGGTGAAAA ATAATCAGCT TAGCGACNAA GAGGGTAGAG
 851    AATTTGATAA CGAAATGACT GCATGCGCCA AACAGAATAN TCCTCAACTG
 901    TGCAGAAAAA ATACTGTAAA AAAGTATCAA AATGTTGCTG ATAAAAGACT
 951    TGCTGCTTCG ATTGCAATAT GTACGGATAT ATCCCGTAGT ACTGAATGTA
1001    GAACAATCAG AAAACAACAT TTGATCGATA GTAGAAGCCT TCATTCATCT
1051    TGGGAAGCAG GTCTAATTGG TAAAGATGAT GAATGGTATA AATTATTCAG
1101    CAAATCTTAC ACCCAAGCAG ATTTGGCTTT ACAGTCTTAT CATTTGAATA
1151    CTGCTGCTAA ATCTTGGCTT CAATCGGGCA ATACAAAGCC TTTATCCGAA
1201    TGGATGTCCG ACCAAGGTTA TACACTTATT TCAGGAGTTA ATCCTAGATT
1251    CATTCCAATA CCAAGAGGGT TTGTAAAACA AAATACACCT ATTACTAATG
1301    TCAAATACCC GGAAGGCATC AGTTTCGATA CAAACCTANA AAGACATCTG
1351    GCAAATGCTG ATGGTTTTAG TCAAGAACAG GGCATTAAAG GAGCCCATAA
1401    CCGCACCAAT NTTATGGCAG AACTAAATTC ACGAGGAGGA NGNGTAAAAT
1451    CTGAAACCCA NACTGATATT GAAGGCATTA CCCGAATTAA ATATGAGATT
1501    CCTACACTAG ACAGGACAGG TAAACCTGAT GGTGGATTTA AGGAAATTTC
1551    AAGTATAAAA ACTGTTTATA ATCCTAAAAA NTTTTNNGAT GATAAAATAC
1601    TTCAAATGGC TCAANATGCT GNTTCACAAG GATATTCAAA AGCCTCTAAA
1651    ATTGCTCAAA ATGAAAGAAC TAAATCAATA TCGGAAAGAA AAAATGTCAT
1701    TCAATTCTCA GAAACCTTTG ACGGAATCAA ATTTAGANNN TATNTNGATG
1751    TAAATACAGG AAGAATTACA AACATTCACC CAGAATAA
```

This encodes a protein having the partial amino acid sequence <SEQ ID 72>:

```
  1  YLKQLQVAKN INWNQVQLAY DRWDYKQEGL TEAGAAIIAL AVTVVTSGAG
 51  TGAVLGLNGA XAAATDAAFA SLASQASVSF INNKGDVGKT LKELGRSSTV
101  KNLVVAAATA GVADKIGASA LXNVSDKQWI NNLTVNLANA GSAALINTAV
151  NGGSLKDXLE ANILAALVNT AHGEAASKIK QLDQHYIVHK IAHAIAGCAA
201  AAANKGKCQD GAIGAAVGEI VGEALTNGKN PDTLTAKERE QILAYSKLVA
251  GTVSGVVGGD VNAAANAAEV AVKNNQLSDX EGREFDNEMT ACAKQNXPQL
301  CRKNTVKKYQ NVADKRLAAS IAICTDISRS TECRTIRKQH LIDSRSLHSS
351  WEAGLIGKDD EWYKLFSKSY TQADLALQSY HLNTAAKSWL QSGNTKPLSE
401  WMSDQGYTLI SGVNPRFIPI PRGFVKQNTP ITNVKYPEGI SFDTNLXRHL
451  ANADGFSQEQ GIKGAHNRTN XMAELNSRGG XVKSETXTDI EGITRIKYEI
```

```
501  PTLDRTGKPD GGFKEISSIK TVYNPKXFXD DKILQMAQXA XSQGYSKASK
551  IAQNERTKSI SERKNVIQFS ETFDGIKFRX YXDVNTGRIT NIHPE*
```

ORF41a and ORF41-1 show 94.8% identity in 595 aa overlap:

```
                                                 10        20        30
orf41a.pep                           YLKQLQVAKNINWNQVQLAYDRWDYKQEGLTEAGAA
                                     |||||||:|::||||||||||:||||||||| ||||
orf41-1          MQVNIQIPYILPRCVRAEDTPYACYLKQLQVTKDVNWNQVQLAYDKWDYKQEGLTGAGAA
                         10        20        30        40        50        60

                  40        50        60        70        80        90
orf41a.pep       IIALAVTVVTSGAGTGAVLGLNGAXAAATDAAFASLASQASVSFINNKGDVGKTLKELGR
                 ||||||||||:|||:||:|||||| ||||||||||||||||||:|||||::|:|||||||
orf41-1          IIALAVTVVTAGAGAGAALGLNGAAAAATDAAFASLASQASVSLINNKGNIGNTLKELGR
                         70        80        90       100       110       120

                 100       110       120       130       140       150
orf41a.pep       SSTVKNLVVAAATAGVADKIGASALXNVSDKQWINNLTVNLANAGSAALINTAVNGGSLK
                 |||||||:||:||||||||||||| |||||||||||||||||||||||||||||||||||
orf41-1          SSTVKNLMVAVATAGVADKIGASALNNVSDKQWINNLTVNLANAGSAALINTAVNGGSLK
                         130       140       150       160       170       180

                 160       170       180       190       200       210
orf41a.pep       DXLEANILAALVNTAHGEAASKIKQLDQHYIVHKIAHAIAGCAAAAANKGKCQDGAIGAA
                 | |||||||||||||||||||||||||||||:||||||||||||||||||||||||||||
orf41-1          DNLEANILAALVNTAHGEAASKIKQLDQHYITHKIAHAIAGCAAAAANKGKCQDGAIGAA
                         190       200       210       220       230       240

                 220       230       240.      250       260       270
orf41a.pep       VGEIVGEALTNGKNPDTLTAKEREQILAYSKLVAGTVSGVVGGDVNAAANAAEVAVKNNQ
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf41-1          VGEIVGEALTNGKNPDTLTAKEREQILAYSKLVAGTVSGVVGGDVNAAANAAEVAVKNNQ
                         250       260'      270       280       290       300

                 280       290       300       310       320       330
orf41a.pep       LSDXEGREFDNEMTACAKQNXPQLCRKNTVKKYQNVADKRLAASIAICTDISRSTECRTI
                 ||| |||||||||||||||| |||||||||||||||||||||||||||||||||||||||
orf41-1          LSDKEGREFDNEMTACAKQNNPQLCRKNTVKKYQNVADKRLAASIAICTDISRSTECRTI
                         310       320       330       340       350       360

                 340       350       360       370       380       390
orf41a.pep       RKQHLIDSRSLHSSWEAGLIGKDDEWYKLFSKSYTQADLALQSYHLNTAAKSWLQSGNTK
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf41-1          RKQHLIDSRSLHSSWEAGLIGKDDEWYKLFSKSYTQADLALQSYHLNTAAKSWLQSGNTK
                         370       380       390       400       410       420

                 400       410       420       430       440       450
orf41a.pep       PLSEWHSDQGYTLISGVNPRFIPIPRGFVKQNTPITNVKYPEGISFDTNLXRHLANADGF
                 |||||||||||||||||||||||||||||||||||||||||||||||||| |||||||||
orf41-1          PLSEWHSDQGYTLISGVNPRFIPIPRGFVKQNTPITNVKYPEGISFDTNLKRHLANADGF
                         430       440       450       460       470       480

                 460       470       480       490       500       510
orf41a.pep       SQEQGIKGAHNRTNXMAELNSRGGXVKSETXTDIEGITRIKYEIPTLDRTGKPDGGFKEI
                 ||:||||||||||| ||||||||| ||||| |||||||||||||||||||||||||||||
orf41-1          SQKQGIKGAHNRTNFMAELNSRGGRVKSETQTDIEGITRIKYEIPTLDRTGKPDGGFKEI
                         490       500       510       520       530       540

                 520       530       540       550       560       570
orf41a.pep       SSIKTVYNPKXFXDDKILQMAQXAXSQGYSKASKIAQNERTKSISERKNVIQFSETFDGI
                 |||||||||| | ||||||||| | |||||||||||||||||||||||||||||||||||
orf41-1          SSIKTVYNPKKFSDDKILQMAQNAASQGYSKASKIAQNERTKSISERKNVIQFSETFDGI
                         550       560       570       580       590       600
```



```
                 580       590
orf41a.pep       KFRXYXDVNTGRITNIHPEX
                 ||| | ||||||||||||||
orf41-1          KFRSYFDVNTGRITNIHPEX
                       610       620
```

Amino acids 25-619 of ORF41-1 were amplified as described above. Figure 6 shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF41-1.

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 17

The following DNA sequence was identified in *N.meningitidis* <SEQ ID 73>

```
  1  ATGGCAATCA TTACATTGTA TTATTCTGTC AATGGTATTT TAAATGTATG
 51  TGCAAAAGCA AAAAATATTC AAGTAGTTGC CAATAATAAG AATATGGTTC
101  TTTTTGGGTT TTTGGamrGC ATCATCGGCG GTTCAACCAA TGCCATGTCT
151  CCCATATTGT TAATATTTTT GCTTAGCGAA ACAGAAAATA AAAATcgTAT
201  CGTAAAATCA AGCAATCTAT GCTATCTTTT GGCGAAAATT GTTCAAATAT
251  ATATGCTAAG AGACCAGTAT TGGTTATTAA ATAAGAGTGA ATACGdTTTA
301  ATATTTTTAC TGTCCGTATT GTCTGTTATT GGATTGTATG TTGGAATTCG
351  GTTAAGGACT AAGATTAGCC CAaATTTTTT TAAAATGTTA ATTTTTATTG
401  tTTTATTGGT ATTGGCtCTG AAAATCGGGC AttCGGGTTT AAtCAAACTT
451  TAA
```

This corresponds to the amino acid sequence <SEQ ID 74; ORF51>:

```
  1  MAIITLYYSV NGILNVCAKA KNIQVVANNK NMVLFGFLXX IIGGSTNAMS
 51  PILLIFLLSE TENKNRIVKS SNLCYLLAKI VQIYNLRDQY WLLNKSEYXL
101  IFLLSVLSVI GLYVGIRLRT KISPNFFKML IFIVLLVLAL KIGHSGLIKL
151  *
```

Further work revealed the complete nucleotide sequence <SEQ ID 75>:

```
  1  ATGCAAGAAA TAATGCAATC TATCGTTTTT GTTGCTGCCG CAATACTGCA
 51  CGGAATTACA GGCATGGGAT TTCCGATGCT CGGTACAACC GCATTGGCTT
101  TTATCATGCC ATTGTCTAAG GTTGTTGCCT TGGTGGCATT ACCAAGCCTG
151  TTAATGAGCT TGTTGGTTCT ATGCAGCAAT AACAAAAAGG GTTTTTGGCA
201  AGAGATTGTT TATTATTTAA AAACCTATAA ATTGCTTGCT ATCGGCAGCG
251  TCGTTGGCAG CATTTTGGGG GTGAAGTTGC TTTTGATACT TCCAGTGTCT
301  TGGCTGCTTT TACTGATGGC AATCATTACA TTGTATTATT CTGTCAATGG
351  TATTTTAAAT GTATGTGCAA AAGCAAAAAA TATTCAAGTA GTTGCCAATA
401  ATAAGAATAT GGTTCTTTTT GGGTTTTTGG CAGGCATCAT CGGCGGTTCA
451  ACCAATGCCA TGTCTCCCAT ATTGTTAATA TTTTTGCTTA GCGAAACAGA
501  AAATAAAAAT CGTATCGTAA AATCAAGCAA TCTATGCTAT CTTTTGGCGA
551  AAATTGTTCA AATATATATG CTAAGAGACC AGTATTGGTT ATTAAATAAG
601  AGTGAATACG GTTTAATATT TTTACTGTCC GTATTGTCTG TTATTGGATT
651  GTATGTTGGA ATTCGGTTAA GGACTAAGAT TAGCCCAAAT TTTTTTAAAA
701  TGTTAATTTT TATTGTTTTA TTGGTATTGG CTCTGAAAAT CGGGCATTCG
751  GGTTTAATCA AACTTTAA
```

This corresponds to the amino acid sequence <SEQ ID 76; ORF51-1>:

```
  1  MQEIMQSIVF VAAAILHGIT GMGFPMLGTT ALAFIMPLSK VVALVALPSL
```

```
 51  LMSLLVLCSN NKKGFWQEIV YYLKTYKLLA IGSVVGSILG VKLLLILPVS
101  WLLLLMAIIT LYYSVNGILN VCAKAKNIQV VANNKNMVLF GFLAGIIGGS
151  TNAMSPILLI FLLSETENKN RIVKSSNLCY LLAKIVQIYM LRDQYWLLNK
201  SEYGLIFLLS VLSVIGLYVG IRLRTKISPN FFKMLIFIVL LVLALKIGHS
251  GLIKL*
```

Computer analysis of this amino acid sequence reveals three putative transmembrane domains. A corresponding ORF from strain A of *N.meningitidis* was also identified:

<u>Homology with a predicted ORF from *N.meningitidis* (strain A)</u>

ORF51 shows 96.7% identity over a 150aa overlap with an ORF (ORF51a) from strain A of *N. meningitidis:*

```
                                                    10        20        30
orf51.pep                                       MAIITLYYSVNGILNVCAKAKNIQVVANNK
                                                ||||||||||||||||||||||||||||||
orf51a       YKLLAIGSVVGSILGVKLLLILPVSWLLLLMAIITLYYSVNGILNVCAKAKNIQVVANNK
                80        90       100       110       120       130

                    40        50        60        70        80        90
orf51.pep    NMVLFGFLXXIIGGSTNAMSPILLIFLLSETENKNRIVKSSNLCYLLAKIVQIYMLRDQY
             ||||||||  ||||||||||||||||||||||||||||:||||||||||||||||||||||
orf51a       NMVLFGFLAGIIGGSTNAMSPILLIFLLSETENKNRIAKSSNLCYLLAKIVQIYMLRDQY
               140       150       160       170       180       190

                   100       110       120       130       140       150
orf51.pep    WLLNKSEYXLIFLLSVLSVIGLYVGIRLRTKISPNFFKMLIFIVLLVLALKIGHSGLIKL
             |||||||| ||||||||||||||||||||||||||||||||||||||||||||:||||||
orf51a       WLLNKSEYGLIFLLSVLSVIGLYVGIRLRTKISPNFFKMLIFIVLLVLALKIGYSGLIKL
               200       210       220       230       240       250
```

ORF51-1 and ORF51a show 99.2% identity in 255 aa overlap:

```
orf51a.pep   MQEIMQSIVFVAAAILHGITGNGFPMLGTTALAFIMPLSKVVALVALPSLLMSLLVLCSN
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf51-1      MQEIMQSIVFVAAAILHGITGNGFPMLGTTALAFIMPLSKVVALVALPSLLMSLLVLCSN

orf51a.pep   NKKGFWQEIVYYLKTYKLLAIGSVVGSILGVKLLLILPVSWLLLLMAIITLYYSVNGILN
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf51-1      NKKGFWQEIVYYLKTYKLLAIGSVVGSILGVKLLLILPVSWLLLLMAIITLYYSVNGILN

orf51a.pep   VCAKAKNIQVVANNKNMVLFGFLAGIIGGSTNAMSPILLIFLLSETENKNRIAKSSNLCY
             |||||||||||||||||||||||||||||||||||||||||||||||||||||:||||||
orf51-1      VCAKAKNIQVVANNKNMVLFGFLAGIIGGSTNAMSPILLIFLLSETENKNRIVKSSNLCY

orf51a.pep   LLAKIVQIYMLRDQYWLLNKSEYGLIFLLSVLSVIGLYVGIRLRTKISPNFFKMLIFIVL
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf51-1      LLAKIVQIYMLRDQYWLLNKSEYGLIFLLSVLSVIGLYVGIRLRTKISPNFFKMLIFIVL

orf51a.pep   LVLALKIGYSGLIKLX
             ||||||||:|||||||
orf51-1      LVLALKIGHSGLIKLX
```

The complete length ORF51a nucleotide sequence <SEQ ID 77> is:

```
  1  ATGCAAGAAA TAATGCAATC TATCGTTTTT GTTGCTGCCG CAATACTGCA
 51  CGGAATTACA GGCATGGGAT TTCCGATGCT CGGTACAACC GCATTGGCTT
101  TTATCATGCC ATTGTCTAAG GTTGTTGCCT TGGTGGCATT ACCAAGCCTG
151  TTAATGAGCT TGTTGGTTCT ATGCAGCAAT AACAAAAAGG GTTTTTGGCA
```



```
201  AGAGATTGTT TATTATTTAA AAACCTATAA ATTGCTTGCT ATCGGCAGCG
251  TCGTTGGCAG CATTTTGGGG GTGAAGTTGC TTTTGATACT TCCAGTGTCT
301  TGGCTGCTTT TACTGATGGC AATCATTACA TTGTATTATT CTGTCAATGG
351  TATTTTAAAT GTATGTGCAA AAGCAAAAAA TATTCAAGTA GTTGCCAATA
401  ATAAGAATAT GGTTCTTTTT GGGTTTTTGG CAGGCATCAT CGGCGGTTCA
451  ACCAATGCCA TGTCTCCCAT ATTGTTAATA TTTTTGCTTA GCGAAACAGA
501  GAATAAAAAT CGTATCGCAA AATCAAGCAA TCTATGCTAT CTTTTGGCAA
551  AAATTGTTCA AATATATATG CTAAGAGACC AGTATTGGTT ATTAAATAAG
601  AGTGAATACG GTTTAATATT TTTACTGTCC GTATTGTCTG TTATTGGATT
651  GTATGTTGGA ATTCGGTTAA GGACTAAGAT TAGCCCAAAT TTTTTTAAAA
701  TGTTAATTTT TATTGTTTTA TTGGTATTGG CTCTGAAAAT CGGGTATTCA
751  GGTTTAATCA AACTTTAA
```

This encodes a protein having amino acid sequence <SEQ ID 78>:

```
  1  MQEIMQSIVF VAAAILHGIT GNGFPMLGTT ALAFIMPLSK VVALVALPSL
 51  LMSLLVLCSN NKKGFWQEIV YYLKTYKLLA IGSVVGSILG VKLLLILPVS
101  WLLLLMAIIT LYYSVNGILN VCAKAKNIQV VANNKNMVLF GFLAGIIGGS
151  TNAMSPILLI FLLSETENKN RIAKSSNLCY LLAKIVQIYM LRDQYWLLNK
201  SEYGLIFLLS VLSVIGLYVG IRLRTKISPN FFKMLIFIVL LVLALKIGYS
251  GLIKL*
```

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 18

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 79>

```
  1  ATGAGACATA TGAAAATACA AAATTATTTA CTAGTATTTA TAGTTTTACA
 51  TATAGCCTTG ATAGTAATTA ATATAGTGTT TGGTTATTTT GTTTTTCTAT
101  TTGATTTTTT TGCGTTTTTG TTTTTTGCAA ACGTCTTTCT TGCTGTAAAT
151  TTATTATTTT TAGAAAAAAA CATAAAAAAC AAATTATTGT TTTTATTGCC
201  GATTTCTATT ATTATATGGA TGGTAATTCA TATTAGTATG ATAAATATAA
251  AATTTTATAA ATTTGAGCAT CAAATAAAGG AACAAAATAT ATCCTCGATT
301  ACTGGGGTGA TAAAACCACA TGATAGTTAT AATTATGTTT ATGACTCAAA
351  TGGATATGCT AAATTAAAAG ATAATCATAG ATATGGTAGG GTAATTAGAG
401  AAACACCTTA TATTGATGTA GTTGCATCTG ATGTTAAAAA TAAATCCATA
451  AGATTAAGCT TGGTTTGTGG TATTCATTCA TATGCTCCAT GTGCCAATTT
501  TATAAAATTT GTCAGG..
```

This corresponds to the amino acid sequence <SEQ ID 80; ORF82>:

```
  1  MRHMKIQNYL LVFIVLHIAL IVINIVFGYF VFLFDFFAFL FFANVFLAVN
 51  LLFLEKNIKN KLLFLLPISI IIWMVIHISM INIKFYKFEH QIKEQNISSI
101  TGVIKPHDSY NYVYDSNGYA KLKDNHRYGR VIRETPYIDV VASDVKNKSI
151  RLSLVCGIHS YAPCANFIKF VR..
```

Further work revealed the complete nucleotide sequence <SEQ ID 81>:

```
  1  ATGAGACATA TGAAAATAAA AAATTATTTA CTAGTATTTA TAGTTTTACA
 51  TATAGCCTTG ATAGTAATTA ATATAGTGTT TGGTTATTTT GTTTTTCTAT
101  TTGATTTTTT TGCGTTTTTG TTTTTTGCAA ACGTCTTTCT TGCTGTAAAT
151  TTATTATTTT TAGAAAAAAA CATAAAAAAC AAATTATTGT TTTTATTGCC
201  GATTTCTATT ATTATATGGA TGGTAATTCA TATTAGTATG ATAAATATAA
251  AATTTTATAA ATTTGAGCAT CAAATAAAGG AACAAAATAT ATCCTCGATT
301  ACTGGGGTGA TAAAACCACA TGATAGTTAT AATTATGTTT ATGACTCAAA
351  TGGATATGCT AAATTAAAAG ATAATCATAG ATATGGTAGG GTAATTAGAG
401  AAACACCTTA TATTGATGTA GTTGCATCTG ATGTTAAAAA TAAATCCATA
```

```
451  AGATTAAGCT TGGTTTGTGG TATTCATTCA TATGCTCCAT GTGCCAATTT
501  TATAAAATTT GCAAAAAAAC CTGTTAAAAT TTATTTTTAT AATCAACCTC
551  AAGGAGATTT TATAGATAAT GTAATATTTG AAATTAATGA TGGAAACAAA
601  AGTTTGTACT TGTTAGATAA GTATAAAACA TTTTTTCTTA TTGAAAACAG
651  TGTTTGTATC GTATTAATTA TTTTATATTT AAAATTTAAT TTGCTTTTAT
701  ATAGGACTTA CTTCAATGAG TTGGAATAG
```

This corresponds to the amino acid sequence <SEQ ID 82; ORF82-1>:

```
  1  MRHMKNKNYL LVFIVLHIAL IVINIVFGYF VFLFDFFAFL FFANVFLAVN
 51  LLFLEKNIKN KLLFLLPISI IIWMVIHISM INIKFYKFEH QIKEQNISSI
101  TGVIKPHDSY NYVYDSNGYA KLKDNHRYGR VIRETPYIDV VASDVKNKSI
151  RLSLVCGIHS YAPCANFIKF AKKPVKIYFY NQPQGDFIDN VIFEINDGNK
201  SLYLLDKYKT FFLIENSVCI VLIILYLKFN LLLYRTYFNE LE*
```

Computer analysis of this amino acid sequence reveals a predicted leader peptide.

A corresponding ORF from strain A of *N.meningitidis* was also identified:

Homology with a predicted ORF from *N.meningitidis* (strain A)

ORF82 shows 97.1% identity over a 172aa overlap with an ORF (ORF82a) from strain A of *N. meningitidis*:

```
                   10        20        30        40        50        60
orf82.pep  MRHMKIQNYLLVFIVLHIALIVINIVFGYFVFLFDFFAFLFFANVFLAVNLLFLEKNIKN
           ||||| :||||||||||||:||||||||||||||||||||||||||||||||||||||||
orf82a     MRHMKNKNYLLVFIVLHITLIVINIVFGYFVFLFDFFAFLFFANVFLAVNLLFLEKNIKN
                   10        20        30        40        50        60

                   70        80        90       100       110       120
orf82.pep  KLLFLLPISIIIWMVIHISMINIKFYKFEHQIKEQNISSITGVIKPHDSYNYVYDSNGYA
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf82a     KLLFLLPISIIIWMVIHISMINIKFYKFEHQIKEQNISSITGVIKPHDSYNYVYDSNGYA
                   70        80        90       100       110       120

                  130       140       150       160       170
orf82.pep  KLKDNHRYGRVIRETPYIDVVASDVKNKSIRLSLVCGIHSYAPCANFIKFVR
           ||||||||||||||||||||||||||||||||||||||||||||||||||::
orf82a     KLKDNHRYGRVIRETPYIDVVASDVKNKSIRLSLVCGIHSYAPCANFIKFAKKPVKIYFY
                  130       140       150       160       170       180
```

ORF82a and ORF82-1 show 99.2% identity in 242 aa overlap:

```
orf82a.pep  MRHMKNKNYLLVFIVLHITLIVINIVFGYFVFLFDFFAFLFFANVFLAVNLLFLEKNIKN
            ||||||||||||||||||:|||||||||||||||||||||||||||||||||||||||||
orf82-1     MRHMKNKNYLLVFIVLHIALIVINIVFGYFVFLFDFFAFLFFANVFLAVNLLFLEKNIKN

orf82a.pep  KLLFLLPISIIIWMVIHISMINIKFYKFEHQIKEQNISSITGVIKPHDSYNYVYDSNGYA
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf82-1     KLLFLLPISIIIWMVIHISMINIKFYKFEHQIKEQNISSITGVIKPHDSYNYVYDSNGYA

orf82a.pep  KLKDNHRYGRVIRETPYIDVVASDVKNKSIRLSLVCGIHSYAPCANFIKFAKKPVKIYFY
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
orf82-1     KLKDNHRYGRVIRETPYIDVVASDVKNKSIRLSLVCGIHSYAPCANFIKFAKKPVKIYFY

orf82a.pep  NQPQGDFIDNVIFEINDGKKSLYLLDKYKTFFLIENSVCIVLIILYLKFNLLLYRTYFNE
            |||||||||||||||||||:||||||||||||||||||||||||||||||||||||||||
orf82-1     NQPQGDFIDNVIFEINDGNKSLYLLDKYKTFFLIENSVCIVLIILYLKFNLLLYRTYFNE
```



```
orf82a.pep  LEX
            |||
orf82-1     LEX
```

The complete length ORF82a nucleotide sequence <SEQ ID 83> is:

```
  1  ATGAGACATA TGAAAAATAA AAATTATTTA CTAGTATTTA TAGTTTTACA
 51  TATAACCTTG ATAGTAATTA ATATAGTGTT TGGTTATTTT GTTTTTCTAT
101  TTGATTTTTT TGCGTTTTTG TTTTTTGCAA ACGTCTTTCT TGCTGTAAAT
151  TTATTATTTT TAGAAAAAAA CATAAAAAAC AAATTATTGT TTTTATTGCC
201  GATTTCTATT ATTATATGGA TGGTAATTCA TATTAGTATG ATAAATATAA
251  AATTTTATAA ATTTGAGCAT CAAATAAAGG AACAAAATAT ATCCTCGATT
301  ACTGGGGTGA TAAAACCACA TGATAGTTAT AATTATGTTT ATGACTCAAA
351  TGGATATGCT AAATTAAAAG ATAATCATAG ATATGGTAGG GTAATTAGAG
401  AAACACCTTA TATTGATGTA GTTGCATCTG ATGTTAAAAA TAAATCCATA
451  AGATTAAGCT TGGTTTGTGG TATTCATTCA TATGCTCCAT GTGCCAATTT
501  TATAAAATTT GCAAAAAAAC CTGTTAAAAT TTATTTTTAT AATCAACCTC
551  AAGGAGATTT TATAGATAAT GTAATATTTG AAATTAATGA TGGAAAAAAA
601  AGTTTGTACT TGTTAGATAA GTATAAAACA TTTTTTCTTA TTGAAAACAG
651  TGTTTGTATC GTATTAATTA TTTTATATTT AAAATTTAAT TTGCTTTTAT
701  ATAGGACTTA CTTCAATGAG TTGGAATAG
```

This encodes a protein having amino acid sequence <SEQ ID 84>:

```
  1  MRHMKNKNYL LVFIVLHITL IVINIVFGYF VFLFDFFAFL FFANVFLAVN
 51  LLFLEKNIKN KLLFLLPISI IIWMVIHISM INIKFYKFEH QIKEQNISSI
101  TGVIKPHDSY NYVYDSNGYA KLKDNHRYGR VIRETPYIDV VASDVKNKSI
151  RLSLVCGIHS YAPCANFIKF AKKPVKIYFY NQPQGDFIDN VIFEINDGKK
201  SLYLLDKYKT FFLIENSVCI VLIILYLKFN LLLYRTYFNE LE*
```

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

Example 19

The following partial DNA sequence was identified in *N.meningitidis* <SEQ ID 85>

```
  1  ..ACCCCCAACA GCGTGACCGT CTTGCCGTCT TTCGGCGGAT TCGGGCGTAC
 51    CGGCGCGACC ATCAATGCAG CAGGCGGGGT CGGCATGACT GCCTTTTCGA
101    CAACCTTAAT TTCCGTAGCC GAGGGCGCGG TTGTAGAGCT GCAGGCCGTG
151    AGAGCCAAAG CCGTCAATGC AACCGCCGCT TGCATTTTTA CGGTCTTGAG
201    TAAGGACATT TTCGATTTCC TTTTTATTTT CCGTTTTCAG ACGGCTGACT
251    TCCGCCTGTA TTTTCGCCAA AGCCATGCCG ACAGCGTGCG CCTTGACTTC
301    ATATTTAAAA GCTTCCGCGC GTGCCAGTTC CAGTTCGCGC GCATAGTTTT
351    GAGCCGACAA CAGCAGGGCT TGCGCCTTGT CGCGCTCCAT CTTGTCGATG
401    ACCGCCTGCA GCTTCGCAAA TGCCGACTTG TAGCCTTGAT GGTGCGACAC
451    AGCCAAGCCC GTGCCGACAA GCGCGATAAT GGCAATCGGT TGCCAGTAAT
501    TCGCCAGCAG TTTCACGAGA TTCATTCTCG ACCTCCTGAC GCTTCACGCT
551    GA
```

This corresponds to the amino acid sequence <SEQ ID 86; ORF124>:

```
  1  ..TPNSVTVLPS FGGFGRTGAT INAAGGVGMT AFSTTLISVA EGAVVELQAV
 51    RAKAVNATAA CIFTVLSKDI FDFLFIFRFQ TADFRLYFRQ SHADSVRLDF
101    IFKSFRACQF QFARIVLSRQ QQGLRLVALR LVDDRLQLRK CRLVALMVRH
151    SQARADKRQN GNRLPVIRQQ FHEIHSRPPD ASR*
```

Computer analysis of this amino acid sequence predicts a transmembrane domain.

Further work revealed the complete nucleotide sequence <SEQ ID 87>:

```
  1  ATGACTGCCT TTTCGACAAC CTTAATTTCC GTAGCCGAGG GCGCGGTTGT
 51  AGAGCTGCAG GCCGTGAGAG CCAAAGCCGT CAATGCAACC GCCGCTTGCA
101  TTTTTACGGT CTTGAGTAAG GACATTTTCG ATTTCCTTTT TATTTTCCGT
151  TTTCAGACGG CTGACTTCCG CCTGTTTTTT CGCCAAAGCC ATGCCGACAG
201  CGTGCGCCTT GACTTCATAT TTTTTAGCTT CCGCGCGTGC CAGTTCCAGT
251  TCGCGCGCAT AGTTTTGAGC CGACAACAGC AGGGCTTGCG CCTTGTCGCG
301  CTCCATCTTG TCGATGACCG CCTGCTGCTT CGCAAATGCC GACTTGTAGC
351  CTTGATGGTG CGACACAGCC AAGCCCGTGC CGACAAGCGC GATAATGGCA
401  ATCGGTTGCC AGTTATTCGC CAGCAGTTTC ACGAGATTCA TTCTCGACCT
451  CCTGACGCTT CACGCTGA
```

This corresponds to the amino acid sequence <SEQ ID 88; ORF124-1>:

```
  1  MTAFSTTLIS VAEGAVVELQ AVRAKAVNAT AACIFTVLSK DIFDFLFIFR
 51  FQTADFRLFF RQSHADSVRL DFIFFSFRAC QFQFARIVLS RQQQGLRLVA
101  LHLVDDRLLL RKCRLVALMV RHSQARADKR DNGNRLPVIR QQFHEIHSRP
151  PDASR*
```

A corresponding ORF from strain A of *N.meningitidis* was also identified:

<u>Homology with a predicted ORF from *N.meningitidis* (strain A)</u>

ORF124 shows 87.5% identity over a 152aa overlap with an ORF (ORF124a) from strain A of *N. meningitidis:*

```
                       10        20        30        40        50        60
orf124.pep    TPNSVTVLPSFGGFGRTGATINAAGGVGMTAFSTTLISVAEGAVVELQAVRAKAVNATAA
                                          ||||||||||||||:|||||| ||||:|||
orf124a                                   MTAFSTTLISVAEGALVELQAVMAKAVNTTAA
                                                  10        20        30

                       70        80        90       100       110       120
orf124.pep    CIFTVLSKDIFDFLFIFRFQTADFRLYFRQSHADSVRLDFIFKSFRACQFQFARIVLSRQ
              ||||||||||||||||||||||||||:|||||||:|||||| |||:   |||| :|||||
orf124a       CIFTVLSKDIFDFLFIFRFQTADFRLFFRQSHADGVRLDFIFFSFRTRLFQFAGVVLSRQ
                    40        50        60        70        80        90

                      130       140       150       160       170       180
orf124.pep    QQGLRLVALHLVDDRLQLRKCRLVALMVRHSQARADKRDNGNRLPVIRQQFHEIHSRPPD
              ||||||||||:::||| ||| ||||||||| |:||||||:||||||||||||||||||||
orf124a       QQGLRLVALHFLNDRLLLRKSRLVALMVRHRQTRADKRDDGNRLPVIRQQFHEIHSRPPD
                   100       110       120       130       140       150

orf124.pep    ASRX
              :
orf124a       VX
```

ORF124a and ORF124-1 show 89.5% identity in 152 aa overlap:

```
orf124-1.pep    MTAFSTTLISVAEGAVVELQAVRAKAVNATAACIFTVLSKDIFDFLFIFRFQTADFRLFF
                ||||||||||||||:|||||| ||||:|||||||||||||||||||||||||||||||||
orf124a         MTAFSTTLISVAEGALVELQAVMAKAVNTTAACIFTVLSKDIFDFLFIFRFQTADFRLFF

orf124-1.pep    RQSHADSVRLDFIFFSFRACQFQFARIVLSRQQQGLRLVALHLVDDRLLLRKCRLVALMV
                ||||||:||||||||||||:  |||| :||||||||||||||:::||||||| |||||||
orf124a         RQSHADGVRLDFIFFSFRTRLFQFAGVVLSRQQQGLRLVALHFLNDRLLLRKSRLVALMV
```



```
orf124-1.pep    RHSQARADKRDNGNRLPVIRQQFHEIHSRPPDASRX
                || |:||||||:||||||||||||||||||||||:
orf124a         RHRQTRADKRDDGNRLPVIRQQFHEIHSRPPDVX
```

The complete length ORF124a nucleotide sequence <SEQ ID 89> is:

```
  1  ATGACCGCCT TTTCGACAAC CTTAATTTCC GTAGCCGAGG GCGCGCTTGT
 51  AGAGCTGCAA GCCGTGATGG CCAAAGCCGT CAATACAACC GCCGCCTGCA
101  TTTTTACGGT CTTGAGTAAG GACATTTTCG ATTTCCTTTT TATTTTCCGT
151  TTTCAGACGG CTGACTTCCG CCTGTTTTTT CGCCAAAGCC ATGCCGACGG
201  CGTGCGCCTT GACTTCATAT TTTTTAGCTT CCGCACGCGC CTGTTCCAGT
251  TCGCGGGCGT AGTTTTGAGC CGACAACAGC AGGGCTTGCG CCTTGTCGCG
301  CTTCATTTTC TCAATGACCG CCTGCTGCTT CGCAAAAGCC GACTTGTAGC
351  CTTGATGGTG CGACACCGCC AAACCCGTGC CGACAAGCGC GATGATGGCA
401  ATCGGTTGCC AGTTATTCGC CAGCAGTTTC ACGAGATTCA TTCTCGACCT
451  CCTGACGTTT GA
```

This encodes a protein having amino acid sequence <SEQ ID 90>:

```
  1  MTAFSTTLIS VAEGALVELQ AVMAKAVNTT AACIFTVLSK DIFDFLFIFR
 51  FQTADFRLFF RQSHADGVRL DFIFFSFRTR LFQFAGVVLS RQQQGLRLVA
101  LHFLNDRLLL RKSRLVALMV RHRQTRADKR DDGNRLPVIR QQFHEIHSRP
151  PDV*
```

ORF124-1 was amplified as described above. Figure 7 shows plots of hydrophilicity, antigenic index, and AMPHI regions for ORF124-1.

Based on this analysis, it is predicted that this protein from *N.meningitidis*, and its epitopes, could be useful antigens for vaccines or diagnostics.

It will be appreciated that the invention has been described by means of example only, and that modifications may be made whilst remaining within the spirit and scope of the invention.

TABLE I – PCR primers

ORF	Primer	Sequence	Restriction sites
ORF 38	Forward Reverse	CGCGGATCCCATATG-TCGCCGCAAAATTCCGA CCCGCTCGAG-TTTTGCCGCGTTAAAAGC	BamHI-NdeI XhoI
ORF 40	Forward Reverse	CGCGGATCCCATATG-ACCGTGAAGACCGCC CCCGCTCGAG-CCACTGATAACCGACAGA	BamHI-NdeI XhoI
ORF 41	Forward Reverse	CGCGGATCCCATATG-TATTTGAAACAGCTCCAAG CCCGCTCGAG-TTCTGGGTGAATGTTA	BamHI-NdeI XhoI
ORF 44	Forward Reverse	GCGGATCCCATATG-GGCACGGACAACCCC CCCGCTCGAG-ACGTGGGGAACAGTCT	BamHI-NdeI XhoI
ORF 51	Forward Reverse	GCGGATCCCATATG-AAAAATATTCAAGTAGTTGC CCCGCTCGAG-AAGTTTGATTAAACCCG	BamHI-NdeI XhoI
ORF 52	Forward Reverse	CGCGGATCCCATATG-TGCCAACCGCAATCCG CCCGCTCGAG-TTTTTCCAGCTCCGGCA	BamHI-NdeI XhoI
ORF 56	Forward Reverse	GCGGATCCCATATG-GTTATCGGAATATTACTCG CCCGCTCGAG-GGCTGCAGAAGCTGG	BamHI-NdeI XhoI
ORF 69	Forward Reverse	CGCGGATCCCATATG-CGGACGTGGTTGGTTTT CCCGCTCGAG-ATATCTTCCGTTTTTTTCAC	BamHI-NdeI XhoI
ORF 82	Forward Reverse	CGCGGATCCGCTAGC-GTAAATTTATTATTTTTAGAA CCCGCTCGAG-TTCCAACTCATTGAAGTA	BamHI-NheI XhoI
ORF 114	Forward Reverse	CGCGGATCCCATATG-AATAAAGGTTTACATCGCAT CCCGCTCGAG-AATCGCTGCACCGGCT	BamHI-NheI XhoI
ORF 124	Forward Reverse	CGCGGATCCCATATG-ACTGCCTTTTCGACA CCCGCTCGAG-GCGTGAAGCGTCAGGA	BamHI-NheI XhoI

TABLE II – Cloning, expression and purification

ORF	PCR/cloning	His-fusion expression	GST-fusion expression	Purification
orf 38	+	+	+	His-fusion
orf 40	+	+	+	His-fusion
orf 41	+	n.d.	n.d.	
orf 44	+	+	+	His-fusion
orf 51	+	n.d.	n.d.	
orf 52	+	n.d.	+	GST-fusion
orf 56	+	n.d.	n.d.	
orf 69	+	n.d.	n.d.	
orf 82	+	n.d.	n.d.	
orf 114	+	n.d.	+	GST-fusion
orf 124	+	n.d.	n.d.	

CLAIMS

1. A protein comprising an amino acid sequence selected from the group consisting of SEQ IDs 2, 4, and 6.

2. A nucleic acid molecule which encodes a protein according to claim 1.

3. A nucleic acid molecule according to claim 2, comprising a nucleotide sequence selected from the group consisting of SEQ IDs 1, 3, and 5.

4. A protein comprising an amino acid sequence selected from the group consisting of SEQ IDs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, and 90.

5. A protein having 50% or greater sequence identity to a protein according to claim 4.

6. A protein comprising a fragment of an amino acid sequence selected from the group consisting of SEQ IDs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, and 90.

7. An antibody which binds to a protein according to any one of claims 4 to 6.

8. A nucleic acid molecule which encodes a protein according to any one of claims 4 to 6.

9. A nucleic acid molecule according to claim 8, comprising a nucleotide sequence selected from the group consisting of SEQ IDs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, and 89.

10. A nucleic acid molecule comprising a fragment of a nucleotide sequence selected from the group consisting of SEQ IDs 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, and 89.

11. A nucleic acid molecule comprising a nucleotide sequence complementary to a nucleic acid molecule according to any one of claims 8 to 10.

12. A nucleic acid molecule comprising a nucleotide sequences having 50% or greater sequence identity to a nucleic acid molecule according to any one of claims 8 to 11.

13. A nucleic acid molecule which can hybridise to a nucleic acid molecule according to any one of claims 8 to 12 under high stringency conditions.

14. A composition comprising a protein, a nucleic acid molecule, or an antibody according to any preceding claim.

15. A composition according to claim 14 being a vaccine composition or a diagn composition.

16. A composition according to claim 14 or claim 15 for use as a pharmaceutical.

17. The use of a composition according to claim 14 in the manufacture of a medicament for the treatment or prevention of infection due to Neisserial bacteria, particularly *Neisseria meningitidis*.

ABSTRACT

The invention provides proteins from *Neisseria meningitidis* (strains A & B), including amino acid sequences, the corresponding nucleotide sequences, expression data, and serological data. The proteins are useful antigens for vaccines, immunogenic compositions, and/or diagnostics.

FIGURE 1

FIG. 1A

FIG. 1B

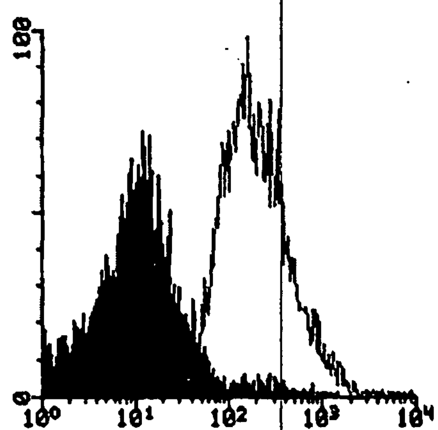


FIG. 1C

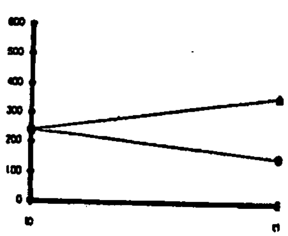
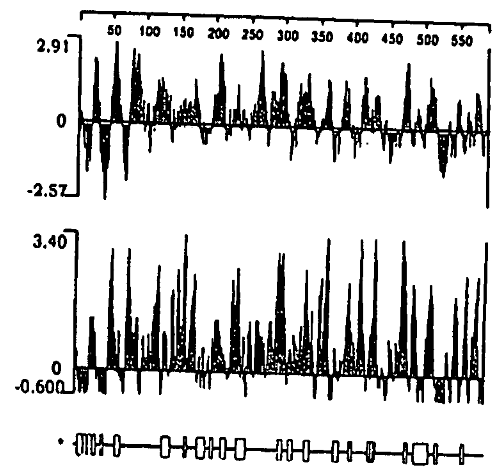


FIG. 1D

FIG 1E



PCT/IB99/00103

FIGURE 2

Fig. 2A

Fig. 2B

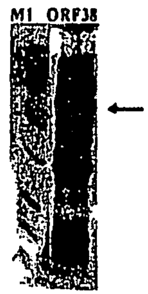
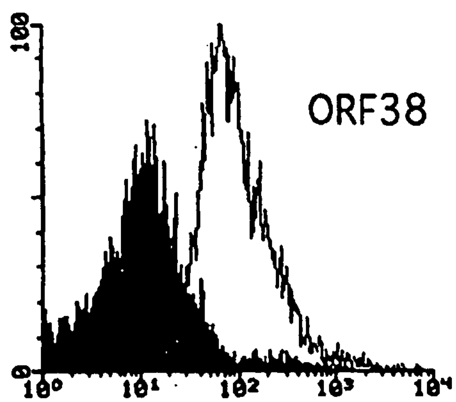


Fig. 2C

Fig. 2D



PCT/IB99/00103

Fig 2E

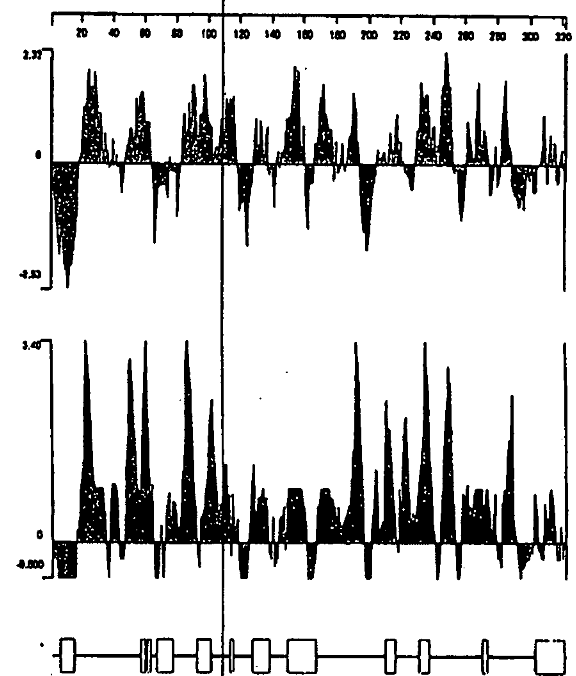


FIGURE 3

FIG. 3A

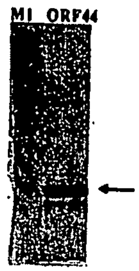


FIG. 3B

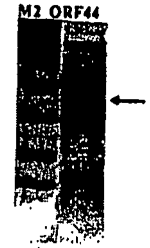


FIG. 3C

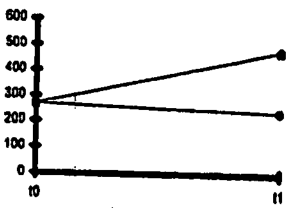


FIG. 3D

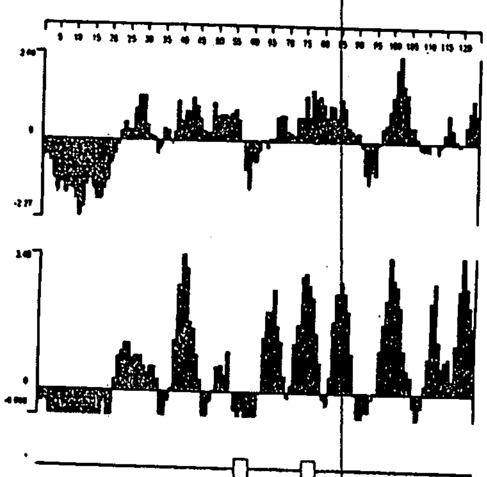


FIGURE 4

FIG. 4A

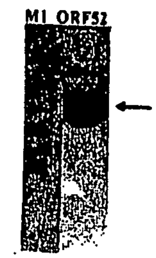


FIG. 4B

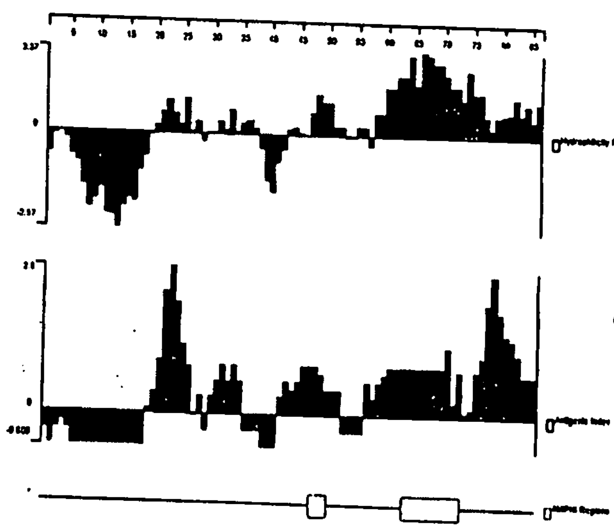


FIGURE 5

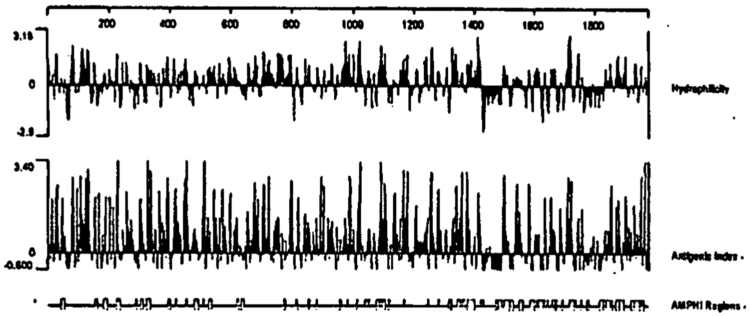


FIGURE 6

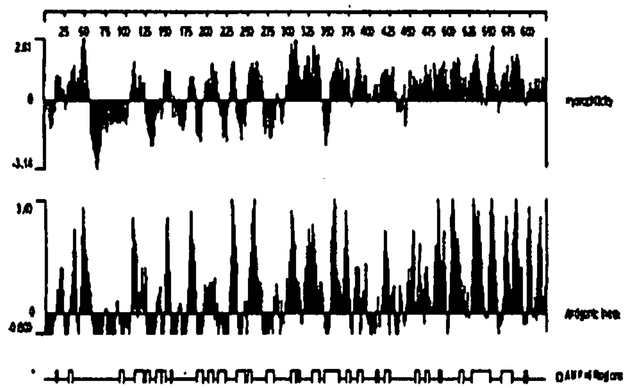
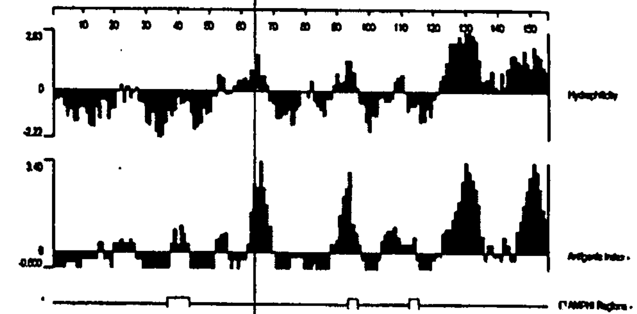


FIGURE 7



CLAIMS

1. A fragment of a protein disclosed in Annex 1, wherein the fragments comprise at least one antigenic determinant.

2. The fragment of claim 1, having a length of 100 amino acids or less.

3. The fragment of claim 1 or claim 2, having a length of 3 amino acids or greater.

4. The fragment of any preceding claim, having an amino acid sequence disclosed in Table I.

5. A polypeptide having 50% or greater sequence identity to the fragment of any preceding claim.

6. A protein comprising one or more fragment of claim 1, claim 2 or claim 3, with the proviso that the protein is not one of the 45 complete protein sequences disclosed in Annex 1.

7. An antibody which recognises the fragment according to any one of claims 1 to 4.

8. A protein comprising a peptide sequence, wherein the peptide sequence is recognised by an antibody according to claim 7.

9. Nucleic acid encoding the fragment of claim 1, claim 2 or claim 3, the polypeptide of claim 5, or the protein of claim 8.

10. A composition comprising the fragment of claim 1, claim 2 or claim 3, the polypeptide of claim 5, the protein of claim 8, the antibody of claim 7, and/or the nucleic acid of claim 9, wherein the composition is a vaccine, a diagnostic reagent, or an immunogenic composition.

11. The composition of claim 10 for use as a medicament

12. The use of the fragment of claim 1, claim 2 or claim 3, the polypeptide of claim 5, the protein of claim 8, the antibody of claim 7, and/or the nucleic acid of claim 9, in the manufacture of (i) a medicament for treating or preventing infection due to Neisserial bacteria (ii) a diagnostic reagent for detecting the presence of Neisserial bacteria or of antibodies raised against Neisserial bacteria and/or (iii) a reagent which can raise antibodies against Neisserial bacteria.

13. A method of treating a patient, comprising administering to the patient a therapeutically effective amount of a composition according to claim 10.